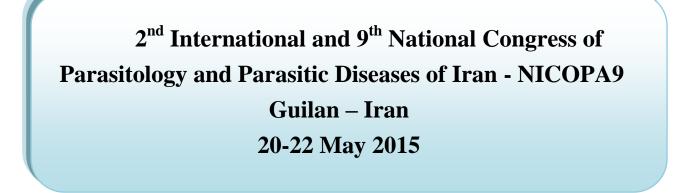






Proceeding of the





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*Iranian Journal of Parasitology (IJP)* is the official publication of Iranian Society of Parasitology (ISP) launched in 2006. The society was inaugurated in 1994 and pursues the improvement of the knowledge on the parasites and parasitic diseases, exchange of scientific knowledge with foreign societies, publicity activities, and consultation on the parasitic diseases, and intimate relationship among society members. IJP is supported and published by Tehran University of Medical Sciences and Health Services and appears quarterly.

The main aims of the Journal are: contribution to the field of **Parasitology**, including all aspects of parasites and parasitic diseases (medical and veterinary) and related fields such as Entomology which may be submitted by scientists from Iran and all over the world.

It is highly appreciated to receive your **Review articles**, **Original papers**, **Short communications**, **Case reports and letters to the Editor** on the above mentioned research fields.

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AbbasAli Eskandarian, Mojtaba Akbari, Gholam-Abbass Jafarnezhad	.303
Abdolali Moshfe, Seyed Abbas Rezaei Naserabad, Seyed Reza Cheraghzade, Nasir Arefkha	ıh,
Roohollah Zare Khafri, Masood Moein, Najmeh Parhizgari	92
Abdollah Rafiei, Ali Jelodar, Mohammad Reza Abbaspor, Mahmud Rahdar, Iran Rashidi	.128
Abdolmajid Fata, Mahmud Parian, Fariba Berengi, Elham Moghaddas, Elham Postchi,	
Parasto Taghzade, Shadi Rahmani	.184
Abdolmajid Fata, Shahab Galavizade, Vida Vakili, Elham Moghaddas	.208
Adel Ebrahimzadeh, Tahereh Davoodi, Abbas Pashaie Naghadeh, Razieh Davoodi	.208
Afsaneh Naderi, Iraj Sharifi, Zahra Babaei, Shahriar Dabiri, Jahangir Mashayekhi, Mehdi	
Bamorovat, Mahshid Mostafavi	.178
Afshin Barazesh, Moradali Fouladvand, Ali Heydari, Jamal Fallahi	.230
Afshin Barazesh, Moradali Fouladvand, Mohammad Rayani, Soleyman Khorrami, Ali Heyd	
	.235
Ahmad Hosseini-Safa, Hossein Yousofi Darani, Mehran Bahadoran Bagh Badorani,	• • -
	.287
Ahmad Hosseini-Safa, Majid Fasihi-Harandi, Mohammad Rostami-Nejad, Amir Ali, Nade	
Pestehchian	.120
Ahmad Karamati, Maryam Niyyati, Samira Dodangeh, Zohreh Lasjerdi	64
Ahmad Mehravaran	.191
Ahmad Reza Esmaeili Rastaghi, Fatemeh Nedaei	.220
Ahmad Reza Esmaeili Rastaghi, Mohsen Asouri, Mohammad Reza Naseri	.109
Ali Eslami, Mohammad Naser Ghorbani, Shima Abdolrahmnai	.282
Ali Farahnak, Seyed Jafar Adnani Sadati, Mohammad Bagher Molaei Rad, Abolfazl Golesta	ıni,
Mohammad Reza Eshraghian	.130
Ali Fattahi Bafghi Yasaman Alijani- and Mustafa Mirza Nejad	92
Ali Fattahi Bafghi, Hossein Hamidi Najat	.300
Ali Haniloo, Negin Torabi, Sommayeh Taheri	.227

Proceedings of 2nd International and 9th National Congress of Parasitology and Parasitic Diseases of Iran (NICOPA 9) Guilan, Iran; 20-22 May 2015

Abbas Hatami, Hossein Hamidinejat, Alireza Alborzi, Hossein Motamedi......151 Abbas Najafi Nasab, Abbas Shahbazi, Esmaeil Fallah, Mahmoud Mahami Oskouei, Shirzad Gholami, Vahid Semnani, Adel Spotin, Ali Shariatzadeh......131 

AbbasAli Eskandarian, Ebrahim sadijadi, Yalda Shokohi, Hosein Ali Yousofi, Marjan

### Author(s)



Page





Guiun, Irun, 20-22 May 2015
Ali Javanshir, Saeid Karimi, Mohmmad Reza Salimi Bejestani, Seyed Mehdi Sarkeshik, Hasan Gafari, Seyed Mohmmad Barani, Mehdi Rafeie Mohmmad, Sina Masomi, Mmehdi
Yazdandoost, Moohsen
Ali khamesipour, Tahereh Rezaei Riabi, Iraj Shrifi, Akram Mir Amin Mohhamadi
Ali Nikpay, Elham Hooshmand
Ali Pakdel, Yasaman Momeni, Pouya Yektaee
Ali Pezeshki, Behnam Mohammadi, Ali Haniloo, Asghar Alejafar, Hafez Mirzanejad
Ali Pouryousef, Amin Asadian, Fariba Berenji, Abdolmajid Fati, Maryam Salehi, Mahmoud
Mahmoudi
Ali Rahiminejad, Shahram Seyedi, Payman Mikayeili, Shahram Khademvatan, Khosrow Hzrati
Tappeh
Ali Rostami, Vahid Fallah Omrani, Maryam Ebrahimi, Zohreh Lasjerdi, Monochehr
Valizadeh, Hamed Behniafar, Amir Reza Javadi, Somayeh Aghamolaie, Seyyed Ahmad
Keramati, Mohhamad Mohsen Homayoni
Ali Yousefi, Iraj Mobedi, Ali sina Karimi
Ali Yousefi, Sadegh Rahbari, Ali sina Karimi, Mohammad Naser Ghorbani232
AliAkbar Shamsian ,Abdolmajid Fata , Abdorrahim Rezaee , Mohammadreza Hedayati-
Moghaddam, Elham Poostchi, Razieh Hosseni Farash, Amin Akbarzadeh, Fatemeh
Sadabadi
AliAkbar Shamsian, Abdolmajid Fata, Abdorrahim Rezaee, Mohammadreza Hedayati-
Moghaddam, Elham Poostchi, Razieh Hosseni Farash, Amin Akbarzadeh, Fatemeh Sadabadi
Aliehsan Heidari, Mehdi Mohebali, Hossein Keshavarz, Kourosh Kabir, Hojatallah Barati,
Behnaz Akhoundi, Homa Hajjaran, Yousef Soultani, Hossein Reisi174
Alireza Alborzi, Leyla Derakhshan, Pedram HaddadMolayan, Omid Hosseininejad, Soroush
Ayubi
Alireza Latifi, Maryam Niyyati, Ali Haghighi
Alireza Mohammadi, Hossein Keshavarz, Saeedeh Shojaee, Mahboobeh Salimi, Mehdi
Mohebali
Amin Ahmadi
Amin Asadian' Ali Pouryousef, Fariba Berenji, Abdolmajid Fati, Maryam Salehi, Mohamad
Reza Mahmoudi, Mohamad Javad Najaf Zadeh
Amin Bagheri, Naser Hoghooghi Rad, Rrasol Madani
Amir Abdoli, Abdolhossein Dalimi, Haleh Soltanghoraee, Fatemeh Ghaffarifar
Amir Abdoli, Abdolhossein Dalimi, Mohsen Arbabi, Majid Pirestani
Amir Hossein Maghsood , Mohammad Fallah , Mohammad Ali Mohaghegh , Hosein Ali
Yousefi, Hossein Yousofi Darani, Mohammad Sadaghian, Rasool Jafari
Amir Keyhani, Amir Tavakoli Kareshk, Hossein Mahmoudvand, Majid Fasihi Harandi 114
Amir Tavakoli Kareshk, Amir Keyhani, Hossein Mahmoudvand, Naser Zia-li
Amir Tavakoli Kareshk, Amir Keyhani, Hossein Mahmoudvand, Naser Zia-Ali
Anahita Razavi Piranshai, Mahdi Tavalla
Anita Mohammadiha, Abdolhossain Dalimi Asl, Mohammadreza Mahmoodi, Mahmood
Parian, Majid Pirestani Mahdi Mohebali
Anoosh Barzigar
Arash Aminpour, Shahram Khademvatan, Shahla Khashave, Firozeh Naseri, Peyman Khezri,
Esmaeil Abasi, Simin Ashtari





Guilan, Iran; 20-22 May 2015
Arash Asadi, Alireza Keyhani, Zahra Babaei, Iraj Sharifi, Majid Fasihi Harandi, Naser Ziaali
Argaw-Dagne, Daniel
Arghavan Vafafar Gholamreza Hatam
Arjang Partoanadazanpor, Zivar Sadeghi Dehkordi, Loghman Akradi
Arman Manochehri, Gghasem Zamini, Mohamad Bagher Khadem Erfan, Ashkan Faridi61
Asad Mirzaei, Mohammad Reza Mahmoudi, Seyed Hosyen Seyedpour60
Asghar Talbalaghi
Ashkan Faridi, Ali Farahnak, Taghi Golmohammadi, Mohammadreza eshraghian, Yosef
sharifi, Mohammadbagher Molaei Rad98
Ashkan Saghatforoush102
Ashkan Saghatforoush
Asma Samiai, Reza Nabavi, Fariborz Shariati Sharifi, Mohammad Jahantigh
Ataollah Hajihassani, Hajir Khooram, Shilan Maroufi, Mahdi Fakhar
Ataollah Hajihassani, Mahdi Fakhar, Shilan Maroufi, Mousa Motavalli haghi, Houjjat
Alizadeh, Abdolsattar Pagheh
Atefe Salehi, Mehran Bahadoran, Seyed Hossein Hejazi, Mehdi Azami, Sepideh Tolouie,
Shervin Ghaffari
Azar Shokri, Shahabedin Sarvi, Mohammad Taghy Rahimi68
Azita Manshoori, Sakineh Mirzaei, Zarrintaj Valadkhani, Mohammad Kazemi Arababadi,
Mohsen Rezaeian, Nahid Zainodini, Reza Bahramabadi, Mohammad Zare-Bidaki
Babak Zarin, Reza Goodarzi, Abbas Hatami, Zahra Rashno155
Bahador Shahriari, Niloofar Bavarsad Ahmadpour, Mohammad Hossein Motazedian, Homa
Hajjaran
Baharak Ghorbanzadeh, Sima Rasti, Farnaz Kheirandish, Ahmad Piroozmand, Gholamabbas
Mousavi
Bahare Sedaghat, Bahador Shahriari, Gholamreza Hatam27
Bahareh Najmi, Mostafa Hosseini, Iraj Mobedi, Bahareh Kamranrashani, Eshrat Beigom Kia
Bahman Khalili, Seifollah Mortezaei, Majid Fazeli
Bahram Nikmanesh, Mohammad Bagher Rokni, Hossein Mirhendi, Eshratbigom Kia, Mahdi
Mohebali, Mitra Sharbatkhori, Mitra Zarebavani141
Bahrooz Naghili, Amirreza Javadi Mamaghani, Mahmoud Mahami Oskouei, Abbas Shahbazi,
Esmaeil Fallah, Majid Khanmohammadi264
Banchob Sripa
Behnam Mohammadi-Ghalehbin, Ali Pezeshki, Firouz Amani Peyman Azghani, Soltan
Asghari, Kimia Rahimi Zahra Alizadeh
Behzad Ghorbanzadeh, Javid Sadraei, Behnaz Ghorbanzadeh, Parvane Atabaki, Mehdi
Mosayebi149
Behzad Ghorbanzadeh, Zahra Eslamirad, Behnaz Ghorbanzadeh, Mojtaba Didedar, Reza
Hajihossein
Berenji
Bibi Razieh Hosseini Farrash, Ali Akbar Shamsian, Abdolmajid Fata, Abdorrahim Rezaee,
Mohammad Reza Hedayati Moghaddam, Elham Poustchi, Amin Akbarzadeh, Fatemeh Sad
Abadi
Bijan Esmaeilnejad, Ghader Jailizade-Amin, Syeed Mostafa Mohammadi219





Guilan, Iran; 20-22 May 2015	
Bijan Esmaeilnejad, Seyyed Meysam Abtahi Froushani, Mostafa Golabi, Jafar Arjmand	
Yamchi, Nasser Hajipour	
Bijan Majidi-Shad	55
Bijan Majidi-Shad, Aisa Pak-Nejad, Afrooz Haghdoost	
David Rollinson	50
Dwight D. Bowman	
Dwight D. Bowman	
Ebrahim Badparva, Farnaz Kheyrandish, Javid Sadraei	
Ebrahim Kazemi, Mehdi Tavalla	
Eelham Moghaddas, Reza Shafiei, Alemeh Molaee, Abbas Jadidoleslami	
Eenayat Darabi, Mohsen Rrahimi, Mehdi Mohseni, Sadjjad Parsipour, Tahereh	
Mohammadzadeh, Hojjat Bostan	
Ehsan Ahmadpour, Soheyla Honary, Hamed Barabadi, Milad Ali Mohammadi, Moham	
Taghi Rahimi, Ahad Alizadeh, Azadeh Mizani	
Ehssan Mozaffari, Jalil Nejati, Mojtaba Beik-Mohammadi, Amir Keyhani	
Eissa Soleymani	
Eissa Soleymani, Shahabeddin Sarvi, Azade Mizani, Mohammad Taghi Rahimi, Davood	
Azamia	
Elahe Ebrahimzade, Roohollah Fattahi, Abbas Gerami Sadeghian, Leila Esmaeili	
Elham Akhlaghi , Mohammad Ali Mohammadi ,Saeid Nasibi , Hosein kamyabi , Mehdi	
Bamorovat, Naser Zia Ali, Majid Fasihi Harandi	
Elham Biabangard-Esfahani, Naseh Maleki-Ravasan, Elahe Biabangard-Esfahani	
Elham Hooshmand, Mohammad-Reza Asadpour, Nadia Taefi, Ali Nikpei	
Elham Hooshmand, Mohammad-Reza Asadpour, Nadia Taefi, Leila Asadpour	
	2.74
Elham Kazemi-Rad, Mehdi Mohebali, Mansour Heidari, Mohammad Bagher khadem-	Erfan
Elham Kazemi-Rad ,Mehdi Mohebali , Mansour Heidari , Mohammad Bagher khadem- ,Homa HajjaranRamtin Hadighi , Sassan Rezaie , Ali Khamesipour , Soroor Charedar	Erfan 37
Elham Kazemi-Rad ,Mehdi Mohebali , Mansour Heidari , Mohammad Bagher khadem- ,Homa HajjaranRamtin Hadighi , Sassan Rezaie , Ali Khamesipour , Soroor Charedar Elham Kordsarkachi, Mehdi Tavalla	Erfan 37 157
Elham Kazemi-Rad ,Mehdi Mohebali , Mansour Heidari , Mohammad Bagher khadem- ,Homa HajjaranRamtin Hadighi , Sassan Rezaie , Ali Khamesipour , Soroor Charedar Elham Kordsarkachi, Mehdi Tavalla Elham Moghaddas, Abdolmajid Fata, Aliakbar Shamsian	Erfan 37 157 153
Elham Kazemi-Rad ,Mehdi Mohebali , Mansour Heidari , Mohammad Bagher khadem- ,Homa HajjaranRamtin Hadighi , Sassan Rezaie , Ali Khamesipour , Soroor Charedar Elham Kordsarkachi, Mehdi Tavalla Elham Moghaddas, Abdolmajid Fata, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian	Erfan 37 157 153 95
Elham Kazemi-Rad ,Mehdi Mohebali , Mansour Heidari , Mohammad Bagher khadem- ,Homa HajjaranRamtin Hadighi , Sassan Rezaie , Ali Khamesipour , Soroor Charedar Elham Kordsarkachi, Mehdi Tavalla Elham Moghaddas, Abdolmajid Fata, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian	Erfan 37 157 153 95 250
Elham Kazemi-Rad ,Mehdi Mohebali , Mansour Heidari , Mohammad Bagher khadem- ,Homa HajjaranRamtin Hadighi , Sassan Rezaie , Ali Khamesipour , Soroor Charedar Elham Kordsarkachi, Mehdi Tavalla Elham Moghaddas, Abdolmajid Fata, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian, Fariba Elham Moghaddas, Fariba Berengi, Aliakbar shamsian, Elham Postchi	Erfan 
Elham Kazemi-Rad ,Mehdi Mohebali , Mansour Heidari , Mohammad Bagher khadem- ,Homa HajjaranRamtin Hadighi , Sassan Rezaie , Ali Khamesipour , Soroor Charedar Elham Kordsarkachi, Mehdi Tavalla Elham Moghaddas, Abdolmajid Fata, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian, Fariba Elham Moghaddas, Fariba Berengi, Aliakbar shamsian, Elham Postchi Elham Moghaddas, Hassan Borji, Aliakbar Shamsian, Abbas Jadidoleslami	Erfan 
Elham Kazemi-Rad ,Mehdi Mohebali , Mansour Heidari , Mohammad Bagher khadem- ,Homa HajjaranRamtin Hadighi , Sassan Rezaie , Ali Khamesipour , Soroor Charedar Elham Kordsarkachi, Mehdi Tavalla Elham Moghaddas, Abdolmajid Fata, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian, Fariba Elham Moghaddas, Fariba Berengi, Aliakbar shamsian, Elham Postchi Elham Moghaddas, Hassan Borji, Aliakbar Shamsian, Abbas Jadidoleslami Elham Moghaddas, Hassan Borji, Syed Hosin Fattahi, Abdolmajid Fata	Erfan 
Elham Kazemi-Rad ,Mehdi Mohebali , Mansour Heidari , Mohammad Bagher khadem- ,Homa HajjaranRamtin Hadighi , Sassan Rezaie , Ali Khamesipour , Soroor Charedar Elham Kordsarkachi, Mehdi Tavalla Elham Moghaddas, Abdolmajid Fata, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian, Fariba Elham Moghaddas, Fariba Berengi, Aliakbar shamsian, Elham Postchi Elham Moghaddas, Hassan Borji, Aliakbar Shamsian, Abbas Jadidoleslami Elham Moghaddas, Hassan Borji, Syed Hosin Fattahi, Abdolmajid Fata Elham Moslemi1, Arsalan jalili2, Amir Izadi3, Nariman Mosaffa4	Erfan 
Elham Kazemi-Rad ,Mehdi Mohebali , Mansour Heidari , Mohammad Bagher khadem- ,Homa HajjaranRamtin Hadighi , Sassan Rezaie , Ali Khamesipour , Soroor Charedar Elham Kordsarkachi, Mehdi Tavalla Elham Moghaddas, Abdolmajid Fata, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian, Fariba Elham Moghaddas, Fariba Berengi, Aliakbar shamsian, Elham Postchi Elham Moghaddas, Hassan Borji, Aliakbar Shamsian, Abbas Jadidoleslami Elham Moghaddas, Hassan Borji, Syed Hosin Fattahi, Abdolmajid Fata Elham Moslemi1, Arsalan jalili2, Amir Izadi3, Nariman Mosaffa4 Elham Yousefi, Shahram Khademvatan, Hajir javaherizadeh, Shahrzad Soltani, Ehsan Y	Erfan 
Elham Kazemi-Rad ,Mehdi Mohebali , Mansour Heidari , Mohammad Bagher khadem- ,Homa HajjaranRamtin Hadighi , Sassan Rezaie , Ali Khamesipour , Soroor Charedar Elham Kordsarkachi, Mehdi Tavalla Elham Moghaddas, Abdolmajid Fata, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian, Fariba Elham Moghaddas, Fariba Berengi, Aliakbar shamsian, Elham Postchi Elham Moghaddas, Hassan Borji, Aliakbar Shamsian, Abbas Jadidoleslami Elham Moghaddas, Hassan Borji, Syed Hosin Fattahi, Abdolmajid Fata Elham Moslemi1, Arsalan jalili2, Amir Izadi3, Nariman Mosaffa4 Elham Yousefi, Shahram Khademvatan, Hajir javaherizadeh, Shahrzad Soltani, Ehsan Y	Erfan 
Elham Kazemi-Rad ,Mehdi Mohebali , Mansour Heidari , Mohammad Bagher khadem- ,Homa HajjaranRamtin Hadighi , Sassan Rezaie , Ali Khamesipour , Soroor Charedar Elham Kordsarkachi, Mehdi Tavalla Elham Moghaddas, Abdolmajid Fata, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian, Fariba Elham Moghaddas, Fariba Berengi, Aliakbar shamsian, Elham Postchi Elham Moghaddas, Hassan Borji, Aliakbar Shamsian, Abbas Jadidoleslami Elham Moghaddas, Hassan Borji, Aliakbar Shamsian, Abbas Jadidoleslami Elham Moghaddas, Hassan Borji, Syed Hosin Fattahi, Abdolmajid Fata Elham Moslemi1, Arsalan jalili2, Amir Izadi3, Nariman Mosaffa4  Elham Yousefi, Shahram Khademvatan, Hajir javaherizadeh, Shahrzad Soltani, Ehsan Y	Erfan 
Elham Kazemi-Rad ,Mehdi Mohebali , Mansour Heidari , Mohammad Bagher khadem- ,Homa HajjaranRamtin Hadighi , Sassan Rezaie , Ali Khamesipour , Soroor Charedar Elham Kordsarkachi, Mehdi Tavalla Elham Moghaddas, Abdolmajid Fata, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian, Fariba Elham Moghaddas, Fariba Berengi, Aliakbar shamsian, Elham Postchi Elham Moghaddas, Hassan Borji, Aliakbar Shamsian, Abbas Jadidoleslami Elham Moghaddas, Hassan Borji, Aliakbar Shamsian, Abbas Jadidoleslami Elham Moghaddas, Hassan Borji, Syed Hosin Fattahi, Abdolmajid Fata Elham Moslemi1, Arsalan jalili2, Amir Izadi3, Nariman Mosaffa4 Elham Yousefi, Shahram Khademvatan, Hajir javaherizadeh, Shahrzad Soltani, Ehsan Y  Elham yousefi, Shahram Khademvatan, Jasem Saki, Roya Salrhi, Ehsan Yousefi Elmira Yazdani, Abbas Jamshidian, Ali Afshari Moghadam, Foruzan Jahangiri, Leila Sat	Erfan 
Elham Kazemi-Rad ,Mehdi Mohebali , Mansour Heidari , Mohammad Bagher khadem- ,Homa HajjaranRamtin Hadighi , Sassan Rezaie , Ali Khamesipour , Soroor Charedar Elham Kordsarkachi, Mehdi Tavalla Elham Moghaddas, Abdolmajid Fata, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian, Fariba Elham Moghaddas, Fariba Berengi, Aliakbar shamsian, Elham Postchi Elham Moghaddas, Hassan Borji, Aliakbar Shamsian, Abbas Jadidoleslami Elham Moghaddas, Hassan Borji, Syed Hosin Fattahi, Abdolmajid Fata Elham Moslemi1, Arsalan jalili2, Amir Izadi3, Nariman Mosaffa4 Elham Yousefi, Shahram Khademvatan, Hajir javaherizadeh, Shahrzad Soltani, Ehsan Y  Elham yousefi, Shahram Khademvatan, Jasem Saki, Roya Salrhi, Ehsan Yousefi Elmira Yazdani, Abbas Jamshidian, Ali Afshari Moghadam, Foruzan Jahangiri, Leila Sat	Erfan 
Elham Kazemi-Rad ,Mehdi Mohebali , Mansour Heidari , Mohammad Bagher khadem- ,Homa HajjaranRamtin Hadighi , Sassan Rezaie , Ali Khamesipour , Soroor Charedar Elham Kordsarkachi, Mehdi Tavalla Elham Moghaddas, Abdolmajid Fata, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian, Fariba Elham Moghaddas, Fariba Berengi, Aliakbar shamsian, Elham Postchi Elham Moghaddas, Hassan Borji, Aliakbar Shamsian, Abbas Jadidoleslami Elham Moghaddas, Hassan Borji, Syed Hosin Fattahi, Abdolmajid Fata Elham Moslemi1, Arsalan jalili2, Amir Izadi3, Nariman Mosaffa4 Elham Yousefi, Shahram Khademvatan, Hajir javaherizadeh, Shahrzad Soltani, Ehsan Y  Elham yousefi, Shahram Khademvatan, Jasem Saki, Roya Salrhi, Ehsan Yousefi Elmira Yazdani, Leila Saravani, Ali Afshari Moghadam, Foruzan Jahangiri	Erfan 
Elham Kazemi-Rad ,Mehdi Mohebali , Mansour Heidari , Mohammad Bagher khadem- ,Homa HajjaranRamtin Hadighi , Sassan Rezaie , Ali Khamesipour , Soroor Charedar Elham Kordsarkachi, Mehdi Tavalla Elham Moghaddas, Abdolmajid Fata, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian, Fariba Elham Moghaddas, Fariba Berengi, Aliakbar shamsian, Elham Postchi Elham Moghaddas, Hassan Borji, Aliakbar Shamsian, Abbas Jadidoleslami Elham Moghaddas, Hassan Borji, Syed Hosin Fattahi, Abdolmajid Fata Elham Moghaddas, Hassan Borji, Syed Hosin Fattahi, Abdolmajid Fata Elham Moghaddas, Hassan Borji, Syed Hosin Fattahi, Abdolmajid Fata Elham Yousefi, Shahram Khademvatan, Hajir javaherizadeh, Shahrzad Soltani, Ehsan Y  Elham yousefi, Shahram Khademvatan, Jasem Saki, Roya Salrhi, Ehsan Yousefi Elmira Yazdani, Leila Saravani, Ali Afshari Moghadam, Foruzan Jahangiri Elnaz Giahi, Mohammad Taghi Ahady, Hashem Yaghoubi	Erfan 
Elham Kazemi-Rad ,Mehdi Mohebali , Mansour Heidari , Mohammad Bagher khadem- ,Homa HajjaranRamtin Hadighi , Sassan Rezaie , Ali Khamesipour , Soroor Charedar Elham Kordsarkachi, Mehdi Tavalla Elham Moghaddas, Abdolmajid Fata, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian, Fariba Elham Moghaddas, Fariba Berengi, Aliakbar shamsian, Elham Postchi Elham Moghaddas, Hassan Borji, Aliakbar Shamsian, Abbas Jadidoleslami Elham Moghaddas, Hassan Borji, Syed Hosin Fattahi, Abdolmajid Fata Elham Moslemi1, Arsalan jalili2, Amir Izadi3, Nariman Mosaffa4 Elham Yousefi, Shahram Khademvatan, Hajir javaherizadeh, Shahrzad Soltani, Ehsan Y  Elham yousefi, Shahram Khademvatan, Jasem Saki, Roya Salrhi, Ehsan Yousefi Elmira Yazdani, Leila Saravani, Ali Afshari Moghadam, Foruzan Jahangiri Elnaz Giahi, Mohammad Taghi Ahady, Hashem Yaghoubi	Erfan 
Elham Kazemi-Rad ,Mehdi Mohebali , Mansour Heidari , Mohammad Bagher khadem- ,Homa HajjaranRamtin Hadighi , Sassan Rezaie , Ali Khamesipour , Soroor Charedar Elham Kordsarkachi, Mehdi Tavalla Elham Moghaddas, Abdolmajid Fata, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian Elham Moghaddas, Fariba Berengi, Aliakbar shamsian, Elham Postchi Elham Moghaddas, Hassan Borji, Aliakbar Shamsian, Abbas Jadidoleslami Elham Moghaddas, Hassan Borji, Syed Hosin Fattahi, Abdolmajid Fata Elham Moghaddas, Hassan Borji, Syed Hosin Fattahi, Abdolmajid Fata Elham Moslemi1, Arsalan jalili2, Amir Izadi3, Nariman Mosaffa4 Elham Yousefi, Shahram Khademvatan, Hajir javaherizadeh, Shahrzad Soltani, Ehsan Y Elham yousefi, Shahram Khademvatan, Jasem Saki, Roya Salrhi, Ehsan Yousefi Elmira Yazdani, Abbas Jamshidian, Ali Afshari Moghadam, Foruzan Jahangiri, Leila Sar Elmira Yazdani, Leila Saravani, Ali Afshari Moghadam, Foruzan Jahangiri Elnaz Giahi, Mohammad Taghi Ahady, Hashem Yaghoubi Elnaz Siahi, Mohammad Taghi Ahady, Hashem Yaghoubi Elnaz Nezami, Mohsen Arbabi <sup>M</sup>	Erfan 
Elham Kazemi-Rad ,Mehdi Mohebali , Mansour Heidari , Mohammad Bagher khadem- ,Homa HajjaranRamtin Hadighi , Sassan Rezaie , Ali Khamesipour , Soroor Charedar Elham Kordsarkachi, Mehdi Tavalla Elham Moghaddas, Abdolmajid Fata, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian, Fariba Elham Moghaddas, Fariba Berengi, Aliakbar shamsian, Elham Postchi Elham Moghaddas, Hassan Borji, Aliakbar Shamsian, Abbas Jadidoleslami Elham Moghaddas, Hassan Borji, Syed Hosin Fattahi, Abdolmajid Fata Elham Moghaddas, Hassan Borji, Syed Hosin Fattahi, Abdolmajid Fata Elham Moslemi1, Arsalan jalili2, Amir Izadi3, Nariman Mosaffa4 Elham Yousefi, Shahram Khademvatan, Hajir javaherizadeh, Shahrzad Soltani, Ehsan Y  Elmira Yazdani, Abbas Jamshidian, Ali Afshari Moghadam, Foruzan Jahangiri, Leila Sar Elmaz Giahi, Mohammad Taghi Ahady, Hashem Yaghoubi Elnaz Giahi, Mohammad Taghi Ahady, Hashem Yaghoubi Elnaz Nezami, Mohsen Arbabi <sup>M</sup>	Erfan 
Elham Kazemi-Rad ,Mehdi Mohebali , Mansour Heidari , Mohammad Bagher khadem- ,Homa HajjaranRamtin Hadighi , Sassan Rezaie , Ali Khamesipour , Soroor Charedar Elham Kordsarkachi, Mehdi Tavalla Elham Moghaddas, Abdolmajid Fata, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian Elham Moghaddas, Aliakbar Shamsian Elham Moghaddas, Fariba Berengi, Aliakbar shamsian, Elham Postchi Elham Moghaddas, Hassan Borji, Aliakbar Shamsian, Abbas Jadidoleslami Elham Moghaddas, Hassan Borji, Syed Hosin Fattahi, Abdolmajid Fata Elham Moghaddas, Hassan Borji, Syed Hosin Fattahi, Abdolmajid Fata Elham Moslemi1, Arsalan jalili2, Amir Izadi3, Nariman Mosaffa4 Elham Yousefi, Shahram Khademvatan, Hajir javaherizadeh, Shahrzad Soltani, Ehsan Y Elham yousefi, Shahram Khademvatan, Jasem Saki, Roya Salrhi, Ehsan Yousefi Elmira Yazdani, Abbas Jamshidian, Ali Afshari Moghadam, Foruzan Jahangiri, Leila Sar Elmira Yazdani, Leila Saravani, Ali Afshari Moghadam, Foruzan Jahangiri Elnaz Giahi, Mohammad Taghi Ahady, Hashem Yaghoubi Elnaz Siahi, Mohammad Taghi Ahady, Hashem Yaghoubi Elnaz Nezami, Mohsen Arbabi <sup>M</sup>	Erfan 





Guilan, Iran; 20-22 May 2015
Eric S. Loker, Ramesh Devkota and Sara V. Brant
Esmaeel Fallah, Abbas Shahbazi, Nasibeh Shahbazi, Ahad Bazmani, Majid Khanmohammadi
Esmaeel Fallah, Abbas Shahbazi, Neda Aboalsoltani, Ahad Bazmani, Majid Khanmohammadi
Esmaeel Fallah, Abbas Shahbazi, Peyman Heydarian, Ardavan Ghazanchaei, Majid
Khanmohammadi, Nasrin Mirsamdi
Esmaeel Fallah, Leyla Mahamioskoii, Mahmud Mahamioskoii, Abdol Rasool Safaiyan229
Esmaeel Fallah, Majid Yazdanjoii Abbas Shahbazi, Bahman Rahimi-Esboei
Esmaeel Fallah, Vahideh Hoshmand, Adileh Shirmohammadi, Mahmud Mahamioskoii,
Mohammadhosein Soroshbarhagi, Mortaza Gojazadeh
Esmaeil fallah, Arash Rasoli, Abbas Shahbazi, Majid khanmohammadi, Fatemeh Hamzavi,
Roghayeh Roshanaei
Esmat Bagheri Garmarodi, Hossein Nahrevanian
Faeze Foroughi-Parvar , Gholam-Reza Hatam , Bahador Sarkari , Eskandar Kamali-Sarvestani
189
Faezeh Hamidi, Mahmoud Mahami Oskouei, Mahnaz Talebi, Mehdi Farhoudi, Aliakbar
Taheraghdam, Tohid Kazemi, Homayoun Sadeghi Bazargani, Esmaeil Fallah
Faezeh Hamidi, Nader Ghabouli Mehrabani, Jalal etemadi, Mahmoud mahami oskouei310
Faranak Firoozfar, Abbas Bahrami, Shahin Siatiri, Maryam Zamani Fard
Fariba Berenji, Abdolmajid Fata, Mahmoud Parian, Maryam Nakhaei, Narges Khazaee80
Fariba Berenji, Abdolmajid Fata, Mahmoud Parian, Maryam Nakhaei, Robabeh Izadi jahanparvar
Fariba Berenji, Abdolmajid Fata, Narges Marvi Moghadam, Parisa Naghibozakerin Meibodi,
Mahmoud Parian
Fariba Jaffary, Mohammad Ali Nilforoushzadeh, Latife Abdellahi, Seifolah Mortazaei
Fariborz Mansour-Ghanaei, Afshin Shafaghi, Mohammadsadegh Fallah
Fariborz Mansour-Ghanaei, Malahat Khalili
Farid Tahvildar Biderouni, Taraneh Ziaeian, Aanita Mihammadiha, Alireza Abadh, Bhram
Kazemi, Mohammad Barati
Farideh Esfandiari, Mahmoud Sadjjadi, Farzaneh
Farnaz Kheirandish, Vahid Fallah Omrani, Soheila Rouhani, Seyed Javad Seyed Tabaee,
Bahram Kazemi
Farnaz Malekifard, Mousa Tavassoli, Mohammad Yakhchali
Farnaz Malekifard, Mousa Tavassoli, Mohammad Yakhchali84
Farnaz Malekifard, Mousa Tavassoli, Mohammad Yakhchali85
Farshid Fayyazjahani, Elham Ahmadnezhad39
Farzad Beikpour, Mohammad Taghi Ahady, Shahram Habibzadeh
Farzad motevali haghi, Mohammad Ahanjan, Masoumeh Eslamifar, Hasan Nikokar, Hosna
Hajati, Mohammadreza Harati, Majid Malekzadeh Shafaroudi, Jamshid Yazdani-Charati90
Farzad Motevali Haghi, Mohammad Ahanjan, Masoumeh Eslamifar, Khalil Akbari
Mohammadi, Hosna Hajati, Jamshid Yazdani Charati, Masumali
Farzad Motevali Haghi, Reza Aghili, Shirzad Gholami, Bahar Salmanian, Hasan Nikookar,
Maryam Khangolzadeh Gerav, Masumeh Eslamifar, Hosna Hajati
Farzad Parsa , hossein vosoghi , Irej Mobedi , reza Godarzy71
Farzad Salehi, Rasool Jafari, Seyedeh Maryam Sharafi, Hossein Ali Yousefi, Hossein Yousofi
Darani





<i>Guilan, Iran; 20-22 May 2015</i>
Farzaneh Biglarnia, Sania Karami, Kavoos Solhjoo, Mohammd Hassan Davami, Hassan
Rezanezhad
Farzaneh Kavarizadeh, Shahram Khademvatan, Mohammad Hossein Feiz-Haddad, Mehdi
Zarean, Babak Vazirianzadeh24
Farzaneh Sedaghat, Mahmoud Sadjjadi, Bahador Shahriari138
Farzaneh Zahabiun, Seyed Mahmoud Sadjjadi, Muhammad Hafiznur Yunus, Anizah
Rahumatullah, Mohammad Hosein Falaki Moghaddam, Syazwan Saidin, Rahmah Noordin.54
Fatemeh Abdolahyar, Iraj Sharifi, Ali Mohamadi Shahryar Dabiri, Simin Shamsi-Meymandi,
Manzomeh Shamsi-Meymandi
Fatemeh Amiri Ardehayi , Nishtman Hemmat Boland Pour , Ali Reza Firoz , Akram Mir Amin
Mohammadi ,Amir Javady,Javad Mossalayi, Ali Khamesipour
Fatemeh Ebrahimifard, Mehdi Fakhar, Mehdi Sharif, Davood Nasrollahpoor Shirvani74
Fatemeh Ezatkhah, Iraj Sharifi
Fatemeh Forooghi, Fatemeh Ghafarifar, Zohreh Sharifi, Abdolhosein Dalimi-ASL, Marjaneh
Aghdasi
Fatemeh Jalousian
Fatemeh Kasaian, Hasan Nayebzadeh, Fatemeh Jalousian, H.Reza Shokrani, S.Hosein
Hoseini
Fatemeh Maleki, Somayeh Sarafpoor146
Fatemeh Namdar, Adel Ebrahimzade, Saeed Mohammadi
Fatemeh Parandin, Fariba Feizi, Amirhossein Maghsood, Mohammad Matini, Arestoo
Roshan, Mohammad Fallah
Fatemeh Sayyahi, Mahboobeh Hoseiny, Hajar Ziaei, Shirzad Gholami74
Fatemeh Talebi
Fattaneh Mikaeili, Hossein Mirhendi, Mehdi Mohebali, Mostafa Hosseini, Zabiholah Zarei,
Zahra Heidari, Eshrat Beigom Kia
Fereshteah Jafaei Nodeh, Maryam Sharafi, Sepideh Tuloee, Hossein Hejazi, Zahra Dorosti,
Hossein Yousofi Darani
Forough Sharifi, Mozafar Hooshangi, Hassan Salehi, Bahram Bagherpour, Pegah Soleimani
Salar, Nasim Adibpour, Mohammad Fatollahzadeh, Paria Alizadeh, Rasool Jafari
Ghanizadeh
Ghasem Hosseini Salekdeh
Ghasem Hosseini, Bahador Shahriari, Mohammad Hosain Motazedian, Abdol Ali Moshfea,
Samaneh Abdolahi
Ghasem Zamini, Fares Bahrami, Bahram Kazemi, Mohamad Bagher Khadem Erfan, Ashkan
Faridi
Ghodratollah Salehi Sangani, Hamed Mirjalali, Mostafa Rezaeian
Gholam Hossein Edrissian
Gholamreza Hatam, Bahador Sarkari, Qasem Asgari, Faeze Foroughi Parvar
Gholamreza Molavi
Gholamreza Molavi
Gholamreza Razmi, Amin Moshiri, Mohsen Maleki ,
Gholamreza Razmi, Leila Danehchin
Gholamreza Razmi, Lena Danenchin
Gilda Eslami, Bahador Hajimohammadi, Amin Zohourtabar, Ali Dehghani, Ahmad Oryan, H
Gilda Esiami, Banador Hajimonammadi, Amin Zonourtabar, Ali Dengnani, Anmad Oryan, H Pourmirzaei Tafti
roummizaet 1alu112





Guilan, Iran; 20-22 May 2015
Habib Jasor Garebagh, Hosein Sobati, Hosein Honari
Habib Mohamadzadeh Hajipirlo, Shiva Hosenzadeh
Habib Mohammadzadeh Hajipirloo, Samad Minaei, Shahram Shahabi, Khosrow
HazratiTappeh, Shahram Seyedi, Naser Zia-Ali
Hadi Mirahmadi, Shirzad Fallahi, VahidI Fallah Omrani, Bahram Kazemi, Ahmad
Mehravaran, Javad Seyyedtabaei
Hadi Zahedi, Soodabe Eynipoor, Maryam Shabani, Sama Rashidi
Hajar Yazdani, Mahboubeh Hadipur, Farnaz Heshmat, Maryam Sharafi, Rasoul Jafari,
Hossein Ali Yousofi, Hossein Yousofi Darani, Akram Sepahvand
Hajar Ziaei Hezarjaribi, Najme Nadali
Hajar Ziaei Hezarjaribi, Najme Nadali
Hajar Ziaei Hezarjaribi, Najme Nadali
Hajar Ziaei, Fatehmeh Sayyahi, Mahboobeh Hoseiny, Shirzad Gholami
Hakim Azizi, Ali Farahnak, Iraj Mobedi, Bagher Molaei Rad42
Hamed Ganjali, Asma Askari, Mehdi Bamorovat, Seyed Hadi Hashemi, Masoud Sargolzaei277
Hamed Kiani, Ali Haghighi, Eznollah Azarghashb
Hamed Kiani, Ali Haghighi, Eznollah Azarghashb, Abbas Solgi, Seyyed Javad Seyyed Tabaei,
Nozhat Zebardast
Hamed Kiani, Ali Haghighi, Eznollah Azarghashb, Abbas Solgi, Seyyed Javad Seyyed Tabaei,
Nozhat Zebardast
Hamid Kassiri, Ebrahim Esteghali77
Hamid Kassiri, Hossein Najafi
Hamid Kassiri, Sakineh Safarpor
Hamidreza Shokrani, Zahra Alizade Sarabi, Hassan Norouzian, Omid Dezfoulian, Shahriar
Yavari
Hassan Abidi, Mostafa Alipour, Mohammad Zoladl, Abdolali Moshfe, Masoud Moghimi234
Hassan Borji, Aboulghasem Naghibi, Mehrab Kamelli
Hassan Borji, Alireza Haghparas, Nushin Mer Soleymani, Mohammad Azizadeh, Mohammad
Hosein Nazem Shirazi
Hassan Borji, Alireza Haghparast, Sima Parandeh, Ahmadreza Movasghi, Mohammad
Khakzad
Hassan Borji, Nona Moradpour, Gholamreza Razmi, Mohsen Maleki, Hossein Kazemi45
Hassan Borji, Sara Ghabdian, Abolghasem Naghibi111
Hassan Khorramian, Mohsen Arbabi, Mahmood Mahami, Mehdi Delavari, Hosein Hooshyar,
Mmohammadreza Asgari
Hassan Nasirian
Hassan Nayebzadeh, Saeid Reza Nourollahi Fard, Mohammad Khalili, Nader Zakian, Majid
Taati245
Hassan Rezanezhad, Mohammad Reza Shokooh, Enaiatollah Shadmand, Nooshin
Mohammadi Nezhad, Zahra Mokhtarian, Arash Fallahi, Hadi Rezai Yazdi, Abbas Ahmadi
Vasmehjani
Hassan Vatandoost
Heidar Heidari, Abdolmajid Mohammadzadeh, Jamal Gharekhani
Helen Hoosh Deghati Fooman, Navid Dinparast Djadid, Vahideh Moin Vaziri, Abbas Ali Raz,
Naseh Maleki Ravasan, Hamzeh Alipour, Seyyed Javad Seyyed Tabaei
Hengameh Zandi, Gilda Eslami, Bahador Hajimohammadi176





Homa Hajjaran, Mitra Mohammadi Bazargani, Mehdi Mohebali, Richard Burchmore,
Ghasem Hosseini Salekdeh, Elham Kazemi-Rad, Mohammad Reza Khoramizadeh47
Hooman Hanifehpour, Vafa Saber
Hooman Rahmati Holasoo, Farokh Behtash, Sepideh Ezhari, Hosseinali Ebrahim Zade
Mosavi, Sara Shokrpoor
Hooman Rahmati Holasoo, Sepideh Ezhari, Mehran Ahmadpoor, Sara Shokrpoor, Farokh
Behtash
Hosain Kamiab, Iraj Sharifi, Hamid Daneshvar, Zhra Mahmmodi192
Hosein Mahmodvand, Saeed Sadooghian, Sareh Jahanbakhsh, Ebrahim Saedi Dezaki, Seyed
Reza Mirbadie141
Hosein Mahmoodvand, Sedreza Mirbadie, Saeed adooghian126
Hoseini
Hossein Abdollahi, Behzad Nasiri Ahmadabad, Mohammadtaghi Rezayati, Mohammad
Kazemi Arababadi, Ali Fattahi BAfghi
Hossein Ali Yousefi
Hossein Elyasi, Elaheh Masoudyar, Sehar Salvari, Ali Hosseinzadeh, Mohammad Shafi
Mojadadi
Hossein Elyasi, Mohamad Shafie Mojadadi, Najmeh Mahmoudabadi, Rahim Golmohamadi,
Melika Laal Kargar, Zohreh Sajadinia
Hossein Ghavami Lahiji, Amin Saeidinia, Ilnaz Tavakoli, Behnaz Rahmati, Omid Salkhori and
Keyhan Ashrafi
Hossein Hamidinejat, Masoud Ghorbanpour, Hedieh Jafari, Pedram HaddadMolayan, Lida
Khaleghi Rostamkolaie
Hossein Hamidinejat, Mohammad Hossein Razi Jalali, Mehdi Pourmehdi, Mehran
Tahmasebi259
Hossein Hejazi, Mehran Bahadoran Bagh Badorani, Parvin Ramezani, Mohsen
Hossein Hejazi, Mehran Bahadoran Bagh Badorani, Parvin Ramezani, Mohsen
Hossein Hejazi, Mehran Bahadoran Bagh Badorani, Parvin Ramezani, Mohsen Ghomashloyan
Hossein Hejazi, Mehran Bahadoran Bagh Badorani, Parvin Ramezani, Mohsen Ghomashloyan
Hossein Hejazi, Mehran Bahadoran Bagh Badorani, Parvin Ramezani, Mohsen Ghomashloyan
Hossein Hejazi, Mehran Bahadoran Bagh Badorani, Parvin Ramezani, MohsenGhomashloyan327Hossein Maghsood, Seyed Sajjad Hasheminasab220Hossein Mahmoudvand, Behrouz Ezatpour, Farnaz Kheirandish197Hossein Nahrevanian, Seyedeh Parisa Jafary, Mahin Farahmand, Fatemeh Sadat Ghasemi .210108Hossein Pazoki-Ghohe, Mahdi Fakhar, Abdol Satar Paghe, Mohsen Kolivand, Mahdi Shrif,171
Hossein Hejazi, Mehran Bahadoran Bagh Badorani, Parvin Ramezani, MohsenGhomashloyan327Hossein Maghsood, Seyed Sajjad Hasheminasab220Hossein Mahmoudvand, Behrouz Ezatpour, Farnaz Kheirandish197Hossein Nahrevanian, Seyedeh Parisa Jafary, Mahin Farahmand, Fatemeh Sadat Ghasemi .21010Hossein Pazoki-Ghohe, Mahdi Fakhar, Abdol Satar Paghe, Mohsen Kolivand, Mahdi Shrif,10
Hossein Hejazi, Mehran Bahadoran Bagh Badorani, Parvin Ramezani, MohsenGhomashloyan327Hossein Maghsood, Seyed Sajjad Hasheminasab220Hossein Mahmoudvand, Behrouz Ezatpour, Farnaz Kheirandish197Hossein Nahrevanian, Seyedeh Parisa Jafary, Mahin Farahmand, Fatemeh Sadat Ghasemi .210Hossein Pazoki-Ghohe, Mahdi Fakhar, Abdol Satar Paghe, Mohsen Kolivand, Mahdi Shrif,Mahdi Mohebali, Ghafur Tavakoli171Hossein Yousofi Darani, Milad Badri Chookami, Seyedeh Maryam Sharafi, Mehran BahadoranBagh Badorani262
Hossein Hejazi, Mehran Bahadoran Bagh Badorani, Parvin Ramezani, MohsenGhomashloyan327Hossein Maghsood, Seyed Sajjad Hasheminasab220Hossein Mahmoudvand, Behrouz Ezatpour, Farnaz Kheirandish197Hossein Nahrevanian, Seyedeh Parisa Jafary, Mahin Farahmand, Fatemeh Sadat Ghasemi .210Hossein Pazoki-Ghohe, Mahdi Fakhar, Abdol Satar Paghe, Mohsen Kolivand, Mahdi Shrif,Mahdi Mohebali, Ghafur Tavakoli171Hossein Yousofi Darani, Milad Badri Chookami, Seyedeh Maryam Sharafi, Mehran Bahadoran
Hossein Hejazi, Mehran Bahadoran Bagh Badorani, Parvin Ramezani, MohsenGhomashloyan327Hossein Maghsood, Seyed Sajjad Hasheminasab220Hossein Mahmoudvand, Behrouz Ezatpour, Farnaz Kheirandish197Hossein Nahrevanian, Seyedeh Parisa Jafary, Mahin Farahmand, Fatemeh Sadat Ghasemi .210Hossein Pazoki-Ghohe, Mahdi Fakhar, Abdol Satar Paghe, Mohsen Kolivand, Mahdi Shrif,Mahdi Mohebali, Ghafur Tavakoli171Hossein Yousofi Darani, Milad Badri Chookami, Seyedeh Maryam Sharafi, Mehran BahadoranBagh Badorani262
Hossein Hejazi, Mehran Bahadoran Bagh Badorani, Parvin Ramezani, MohsenGhomashloyan327Hossein Maghsood, Seyed Sajjad Hasheminasab220Hossein Mahmoudvand, Behrouz Ezatpour, Farnaz Kheirandish197Hossein Nahrevanian, Seyedeh Parisa Jafary, Mahin Farahmand, Fatemeh Sadat Ghasemi .210Hossein Pazoki-Ghohe, Mahdi Fakhar, Abdol Satar Paghe, Mohsen Kolivand, Mahdi Shrif,Mahdi Mohebali, Ghafur Tavakoli171Hossein Yousofi Darani, Milad Badri Chookami, Seyedeh Maryam Sharafi, Mehran BahadoranBagh Badorani262Hossin forughi, Nasrollah Vahedi Noori, Mahmoud Golchin147
Hossein Hejazi, Mehran Bahadoran Bagh Badorani, Parvin Ramezani, MohsenGhomashloyan327Hossein Maghsood, Seyed Sajjad Hasheminasab220Hossein Mahmoudvand, Behrouz Ezatpour, Farnaz Kheirandish197Hossein Nahrevanian, Seyedeh Parisa Jafary, Mahin Farahmand, Fatemeh Sadat Ghasemi .210Hossein Pazoki-Ghohe, Mahdi Fakhar, Abdol Satar Paghe, Mohsen Kolivand, Mahdi Shrif,Mahdi Mohebali, Ghafur Tavakoli171Hossein Yousofi Darani, Milad Badri Chookami, Seyedeh Maryam Sharafi, Mehran BahadoranBagh Badorani262Hossin forughi, Nasrollah Vahedi Noori, Mahmoud Golchin147Iraj Shrifi, Tahereh Rezaei Riabi, Akram Mir Amin Mohhamadi, Ali khamesipour207
Hossein Hejazi, Mehran Bahadoran Bagh Badorani, Parvin Ramezani, MohsenGhomashloyan327Hossein Maghsood, Seyed Sajjad Hasheminasab220Hossein Mahmoudvand, Behrouz Ezatpour, Farnaz Kheirandish197Hossein Nahrevanian, Seyedeh Parisa Jafary, Mahin Farahmand, Fatemeh Sadat Ghasemi .210Hossein Pazoki-Ghohe, Mahdi Fakhar, Abdol Satar Paghe, Mohsen Kolivand, Mahdi Shrif,Mahdi Mohebali, Ghafur Tavakoli171Hossein Yousofi Darani, Milad Badri Chookami, Seyedeh Maryam Sharafi, Mehran BahadoranBagh Badorani262Hossin forughi, Nasrollah Vahedi Noori, Mahmoud Golchin147Iraj Shrifi, Tahereh Rezaei Riabi, Akram Mir Amin Mohhamadi, Ali khamesipour207J Parvaneh, A Alborzi, M.h Razijalali, M Jadidi76
Hossein Hejazi, Mehran Bahadoran Bagh Badorani, Parvin Ramezani, MohsenGhomashloyan327Hossein Maghsood, Seyed Sajjad Hasheminasab220Hossein Mahmoudvand, Behrouz Ezatpour, Farnaz Kheirandish197Hossein Nahrevanian, Seyedeh Parisa Jafary, Mahin Farahmand, Fatemeh Sadat Ghasemi .210Hossein Pazoki-Ghohe, Mahdi Fakhar, Abdol Satar Paghe, Mohsen Kolivand, Mahdi Shrif,Mahdi Mohebali, Ghafur Tavakoli171Hossein Yousofi Darani, Milad Badri Chookami, Seyedeh Maryam Sharafi, Mehran BahadoranBagh Badorani262Hossin forughi, Nasrollah Vahedi Noori, Mahmoud Golchin147Iraj Shrifi, Tahereh Rezaei Riabi, Akram Mir Amin Mohhamadi, Ali khamesipour207J Parvaneh, A Alborzi, M.h Razijalali, M Jadidi76Jafar Adnani Sadati, Ali Farahnak, Mohammad Bagher Molaei Rad, Abolfazl Golestani,
Hossein Hejazi, Mehran Bahadoran Bagh Badorani, Parvin Ramezani, MohsenGhomashloyan327Hossein Maghsood, Seyed Sajjad Hasheminasab220Hossein Mahmoudvand, Behrouz Ezatpour, Farnaz Kheirandish197Hossein Nahrevanian, Seyedeh Parisa Jafary, Mahin Farahmand, Fatemeh Sadat Ghasemi .210Hossein Pazoki-Ghohe, Mahdi Fakhar, Abdol Satar Paghe, Mohsen Kolivand, Mahdi Shrif,Mahdi Mohebali, Ghafur Tavakoli.171Hossein Yousofi Darani, Milad Badri Chookami, Seyedeh Maryam Sharafi, Mehran BahadoranBagh Badorani262Hossin forughi, Nasrollah Vahedi Noori, Mahmoud Golchin147Iraj Shrifi, Tahereh Rezaei Riabi, Akram Mir Amin Mohhamadi, Ali khamesipour207J Parvaneh, A Alborzi, M.h Razijalali, M Jadidi76Jafar Adnani Sadati, Ali Farahnak, Mohammad Bagher Molaei Rad, Abolfazl Golestani, Mohammad Reza Eshraghian, Iraj Mobedi, Mehdi Mohebali113
Hossein Hejazi, Mehran Bahadoran Bagh Badorani, Parvin Ramezani, MohsenGhomashloyan327Hossein Maghsood, Seyed Sajjad Hasheminasab220Hossein Mahmoudvand, Behrouz Ezatpour, Farnaz Kheirandish197Hossein Nahrevanian, Seyedeh Parisa Jafary, Mahin Farahmand, Fatemeh Sadat Ghasemi .210Hossein Pazoki-Ghohe, Mahdi Fakhar, Abdol Satar Paghe, Mohsen Kolivand, Mahdi Shrif,Mahdi Mohebali, Ghafur Tavakoli.171Hossein Yousofi Darani, Milad Badri Chookami, Seyedeh Maryam Sharafi, Mehran BahadoranBagh Badorani262Hossin forughi, Nasrollah Vahedi Noori, Mahmoud Golchin147Iraj Shrifi, Tahereh Rezaei Riabi, Akram Mir Amin Mohhamadi, Ali khamesipour207J Parvaneh, A Alborzi, M.h Razijalali, M Jadidi.76Jafar Adnani Sadati, Ali Farahnak, Mohammad Bagher Molaei Rad, Abolfazl Golestani,113Jalil Nejati, Malek Kiyani, Mohammad Sakeni, Ehssan Mozaffari.221
Hossein Hejazi, Mehran Bahadoran Bagh Badorani, Parvin Ramezani, MohsenGhomashloyan327Hossein Maghsood, Seyed Sajjad Hasheminasab220Hossein Mahmoudvand, Behrouz Ezatpour, Farnaz Kheirandish197Hossein Nahrevanian, Seyedeh Parisa Jafary, Mahin Farahmand, Fatemeh Sadat Ghasemi .210Hossein Pazoki-Ghohe, Mahdi Fakhar, Abdol Satar Paghe, Mohsen Kolivand, Mahdi Shrif,Mahdi Mohebali, Ghafur Tavakoli171Hossein Yousofi Darani, Milad Badri Chookami, Seyedeh Maryam Sharafi, Mehran BahadoranBagh Badorani262Hossin forughi, Nasrollah Vahedi Noori, Mahmoud Golchin147Iraj Shrifi, Tahereh Rezaei Riabi, Akram Mir Amin Mohhamadi, Ali khamesipour207J Parvaneh, A Alborzi, M.h Razijalali, M Jadidi76Jafar Adnani Sadati, Ali Farahnak, Mohammad Bagher Molaei Rad, Abolfazl Golestani,113Mohammad Reza Eshraghian, Iraj Mobedi, Mehdi Mohebali113Jalil Nejati, Malek Kiyani, Mohammad Sakeni, Ehssan Mozaffari221Jalil Nejati, Seyyed Hassan Moosa-Kazemi, Ehssan Mozaffari90
Hossein Hejazi, Mehran Bahadoran Bagh Badorani, Parvin Ramezani, MohsenGhomashloyan327Hossein Maghsood, Seyed Sajjad Hasheminasab220Hossein Mahmoudvand, Behrouz Ezatpour, Farnaz Kheirandish197Hossein Nahrevanian, Seyedeh Parisa Jafary, Mahin Farahmand, Fatemeh Sadat Ghasemi .210Hossein Pazoki-Ghohe, Mahdi Fakhar, Abdol Satar Paghe, Mohsen Kolivand, Mahdi Shrif,Mahdi Mohebali, Ghafur Tavakoli171Hossein Yousofi Darani, Milad Badri Chookami, Seyedeh Maryam Sharafi, Mehran BahadoranBagh Badorani262Hossin forughi, Nasrollah Vahedi Noori, Mahmoud Golchin147Iraj Shrifi, Tahereh Rezaei Riabi, Akram Mir Amin Mohhamadi, Ali khamesipour207J Parvaneh, A Alborzi, M.h Razijalali, M Jadidi76Jafar Adnani Sadati, Ali Farahnak, Mohammad Bagher Molaei Rad, Abolfazl Golestani,113Jalil Nejati, Malek Kiyani, Mohammad Sakeni, Ehssan Mozaffari221Jalil Nejati, Seyyed Hassan Moosa-Kazemi, Ehssan Mozaffari90Jamal Gharekhani309
Hossein Hejazi, Mehran Bahadoran Bagh Badorani, Parvin Ramezani, MohsenGhomashloyan327Hossein Maghsood, Seyed Sajjad Hasheminasab220Hossein Mahmoudvand, Behrouz Ezatpour, Farnaz Kheirandish197Hossein Nahrevanian, Seyedeh Parisa Jafary, Mahin Farahmand, Fatemeh Sadat Ghasemi .210Hossein Pazoki-Ghohe, Mahdi Fakhar, Abdol Satar Paghe, Mohsen Kolivand, Mahdi Shrif,Mahdi Mohebali, Ghafur Tavakoli171Hossein Yousofi Darani, Milad Badri Chookami, Seyedeh Maryam Sharafi, Mehran BahadoranBagh Badorani262Hossin forughi, Nasrollah Vahedi Noori, Mahmoud Golchin147Iraj Shrifi, Tahereh Rezaei Riabi, Akram Mir Amin Mohhamadi, Ali khamesipour207J Parvaneh, A Alborzi, M.h Razijalali, M Jadidi76Jafar Adnani Sadati, Ali Farahnak, Mohammad Bagher Molaei Rad, Abolfazl Golestani,113Mohammad Reza Eshraghian, Iraj Mobedi, Mehdi Mohebali113Jalil Nejati, Malek Kiyani, Mohammad Sakeni, Ehssan Mozaffari221Jamal Gharekhani309Jamal Gharekhani315
Hossein Hejazi, Mehran Bahadoran Bagh Badorani, Parvin Ramezani, Mohsen327Ghomashloyan327Hossein Maghsood, Seyed Sajjad Hasheminasab220Hossein Mahmoudvand, Behrouz Ezatpour, Farnaz Kheirandish197Hossein Nahrevanian, Seyedeh Parisa Jafary, Mahin Farahmand, Fatemeh Sadat Ghasemi .210Hossein Pazoki-Ghohe, Mahdi Fakhar, Abdol Satar Paghe, Mohsen Kolivand, Mahdi Shrif,Mahdi Mohebali, Ghafur Tavakoli.171Hossein Yousofi Darani, Milad Badri Chookami, Seyedeh Maryam Sharafi, Mehran BahadoranBag Badorani262Hossin forughi, Nasrollah Vahedi Noori, Mahmoud Golchin147Iraj Shrifi, Tahereh Rezaei Riabi, Akram Mir Amin Mohhamadi, Ali khamesipour207J Parvaneh, A Alborzi, M.h Razijalali, M Jadidi.76Jafar Adnani Sadati, Ali Farahnak, Mohammad Bagher Molaei Rad, Abolfazl Golestani,Mohammad Reza Eshraghian, Iraj Mobedi, Mehdi Mohebali113Jalil Nejati, Malek Kiyani, Mohammad Sakeni, Ehssan Mozaffari221Jamal Gharekhani309Jamal Gharekhani, Abbas Geramisadeghian246





Guuan, Iran; 20-22 May 2015	
Jamal Gharekhani, Heidar Heidari, Mohammadreza Youssefi	
Jamal Gharekhani, Hesamedin Akbarein	
Jasem Saki, Azadeh sepahvand, Farzaneh kavarizadeh, Shahram Khademvatan	338
Jasem Saki, Shahla Shafiee	312
Javad Khedri, Omid Ghashghaei, Forouzan Jahangiri, Ali Afsharimoghaddam	88
Jean Dupouy-Camet & Hélène Yera	
Jean Dupouy-Camet	47
Kambiz Yaghoobi	258
Kamran Akbarzadeh	22
Karimi S., Salimi-Bejestani M.R., Gavanshir A, Sarkeshik S.M, Jafari H. Barani S.M., Rafei	E.
Mohmmadi, Masomi S, Yazdandoost M, Hoseini M	132
Kavous Solhjoo, Morteza Pourahmad, Vahideh Orefaee	320
Keyhan Ashrafi, Maria Adela Valero, Raquel V. Peixoto, Patricio Artigas, Moroslava Pano	va,
Santiago Mas-coma	
Keyhan Ashrafi	
Keyhan Ashrafi, Ali Tahbaz, Meysam Sharifdini, Santiago Mas-Coma	
Keyhan Ashrafi, Meysam Sharifdini, Eshratbeigom Kia, Fataneh Mikaeili	292
Keyhan Ashrafi, Meysam Sharifdini, Mohammad Reza Mahmoudi, Behnaz Rahmati, Zahra	
Heidari, Eshratbeigom Kia, Fataneh Mikaeili	288
Khadijeh Khanaliha, Hamed Mirjalali, Mehdi Mohebali, Fatemeh Tarighi, Mostafa Rezaeia	
	228
Khadijeh Khanaliha, Mostafa Rezaeian, Mehdi Mohebali, Setareh Davoudi, Omid	
RezaHosseini	159
Khojasteh Sharifi-Sarasiabi, Ali Haghighi, Bahram Kazemi, Niloofar Taghipour, Ehsan	
Nazemalhosseini Mojarad, Latif Gachkar	
Khosrow Hazrati Tappeh, Shahram seyedy, Mahmood Ghaderi, Peyman Mikaili	214
Kkoroush Manoucheri Naeni, Sayyed Hadi Ahmadi, Seifollah Mortazaee Mortazaee,	
Mahmoud Rafieian Koopaee	
Kobra Amiri, Mohammad Hadi Nematollahi, Ali Khamesipour, Saeid Nasibi Davarani, Maj	
	134
Kobra Amiri, Mohammad Hadi Nematollahi, Mitra Mehrabani, Saeid Nasibi Davarani, Ma	·
Fasihi Harandi	133
Kobra Mokhtarian, Lame Akhlaghi, Reza Falak, Elham Razmjou, Ahmad Reza Meamar,	
Samaneh Gholami, Kourosh Manouchehri naeini	91
Komeil Mazhab-Jafari, Ali Asghar Valipour, Azimeh Karimyan Firooz Shahrivar, Somaye	• • •
Azimi.	261
Komeil Mazhab-jafari, Ali Asghar Valipour, Azimeh Karimyan, Dariush Bahrami, Firooz	2(1
Shahrivar, Somaye Azimi	
Komeil Mazhab-jafari, Maryam Fasihi, Rrazieh Bahrami, Fateme Moghimi-Zare	
Koroush Manouchehri Naeini, Mojtaba Jaffari, Soleiman Kheiri	
Laila Abasian, Shanaz Shirbazou	
Leila Danehchin, Gholamreza Razmi	302
Leyla Derakhshan, Pedram HaddadMolayan, Lida Khaleghi Rostamkolaei, Moshirifar	272
Ameneh	
Lida Khaleghi Rostamkolayi, Hossein Hamidinejat, Ali Shahriary, Mahmoud Rahdar	
Maedeh Majidi-Shad, Bijan Majidi-Shad, Hooman Hashemian	160





Guilan, Iran; 20-22 May 2015
Mahboubeh Hadipur, Hajar Yazdani, Farnaz Heshmat, Maryam sharafi, Behnam Sanei,
Hossein Yousofi Darani
Mahdi Fakhar, Mehdi karamian, Hossein Pazoki ghoheh, Abdolsattar Pagheh , Sayed
Abobakar Rasooli , Abolsattar Mohib , Hajar Ziaei
Mahdi Mosayebi, Reza Hajihossein, Mojtaba Didehdar, Zahra Eslamirad70
Mahdi Parsaei, Saber Alizadeh, Sedigeh Sarafraz, Mohamad Fathalahzaeh, JafarSadegh
Tabrizi, Ali Mohamadi Alireza Hasanzadeh, Ahad Shahnami, Abbas Shahbazi, Mahmood
Mahami Oskuei
Mahin Farahmand, Hossein Nahrevanian, Vahid Khalaj, Mehdi Mohebali, Ghader Khalili,
Sanaz Naderi
Mahmoud Agholi, Sharbanoo Naderi Shahabadi, Gholam Reza Hatam, Mohammad Hossein
Motazedian
Mahmoud Bahmani, Behrouz Ezatpour
Mahmoud Bahmani, Behrouz Ezatpour
Mahmoud Mahami Oskouei, Esmaeil Fallah, Mahmoud Ahmadi, Abdolrasoul Safaiyan, Salar
Bakhtiyari, Razi Naserifar
Mahmoud Mahami Oskouei, Faezeh Hamidi, Mahnaz Talebi, Mehdi Farhoudi, Aliakbar
Taheraghdam, Tohid Kazemi, Homauoun Sadeghi Bazargani, Esmaeil fallah
Mahmoud Mahami Oskouei, Nader Ghabouli Mehrabani, Abolfazl Miahipour, Esmaeil Fallah,
Abbas Shahbazi, Nahideh Mazhari, Faezeh Hamidi
Mahmoud Mahami Oskouei, Nader Ghabouli Mehrabani, Abolfazl Miahipour, Esmaeil Fallah
Mahsa Esmaeilifallah, Javid Sadraei, Majid Pirestani, Reza Kalantari
Mahsasadat Makki, Iraj Mobedi, Zahra Heidari Soureshjani, Abolfazl Aali, Thomas Stollner,
Mostafa Rezaeian, Nicole Boenke, Gholamreza Mowlavi
Mahshid Mostafavi, Gholamreza Asadikaram, Iraj Sharifi, Sina Kakooei, Nozar Nakhaee 179
Mahshid Mostafavi, Saeideh Farajzadeh, Sana Eybpoosh, Amireh Heshmatkhah, Simin
Shamsi Meymandi
Mahshid Shakibapour, Seyed Hossein Hejazi, Mohsen Mahmoodi, Fatemeh Rostami169
Majid Fasihi Harandi, Mohammad Ali Mohammadi, Balal Sadeghi
Majid Mansouri, Bahador Shahriari, Gholamreza Molavi, Mohsen Najjari, Kambiz Yagoobi .73
manoochehr shabani ,kavous solhjoo ,Aminolah Saadat nia2 , Masoud Esmi110
Manoochehr Shabani, Kavous Solhjoo, Aminolah Saadat nia, Masoud Esmi110
Manouchehr Valizadeh
Mansooreh Dehghani, Mohammad Ali Mohammadi, Seyed Reza Mirbadie, Sima Rostami,
Majid Fasihi Harandi
Mansour Ebrahimi, Amin Ahmadi, Karim Eghdami, Parviz Kheibari161
Mansour Ebrahimi, Amin Ahmadi, Parviz Kheibari, Karim Eghdami
Mansour Ebrahimi, Keivan Adinehbeigi, Kia Bahramnejad, Khatereh Rahmani
Marayam Hakimi Parizi, Dariush Ghaffari, Mohammad Reza Yaghoobi Ershadi, Iraj Sharifi,
Amir Ahmad Akhavan Mehdi Bamorovat
Marjan Sabzevari, Mehdi Tavalla
Marjaneh Aghdasi, Fatemeh Ghafarifar, Abolhossein Dalimi-Asl, Zohreh Sharifi, Fatemeh
Forooghi





Guilan, Iran; 20-22 May 2015
Maryam Ahmadi, Eshrat Beigom Kia, Mostafa Rezaeian Mostafa Hosseini Fateme Tarighi,
Bahare Kamranrashani271
Maryam Alsadat Mirdamadi, Hooman Rahmati-Holasoo, Hosseinali Ebrahimzadeh-Mousavi,
Seyed-Saeed Mirzargar, Mina Farazandeh-Mehr
Maryam Barkhori Mahni, Ahmad Raeisi, Masoomeh Ghaderipour, Nnajmeh Bahrami,
Asadullah AhmadiKahnali
Maryam Barkhori, Mostafa Rezaeian, Eshrat Bigom Kia, Ahmad Raeisi, Khadijeh Khanaliha,
Fatemeh Tarighi, Bahare Kamranrashani
Maryam Ebrahimi, Soheyla Rouhani, Ali rostami, Shirzad Falahi, Soudabe Heydari, Hameh
Behniafar, Mohammad Mohsen Homayoni
Maryam Ganjali, Mahdie Mishmast, Rreza Nabavi
Maryam Ganjali, Mojtaba Keighobadi
Maryam Ganjali, Mojtaba Keighobadi81
Maryam Ghavami, Gilda Eslami, Morteza Vakil, Hamid Nadri, Alireza Moradi, Alireza
Foroumadi175
Maryam Ghobadi Tara, Mahdi Fakhar, Mehdi Sharif, Mehdi Karamian, Reza Bastani, Shaban
Gohardehi
Maryam Hedayati, Reza Kheyrandish, Somayeh Mollae Saghin, Mohammad Hosein Radfar,
Zahra Asadi
Maryam Mahdi, Homa Hajjaran, Mehdi Mohebali, Katayoun Samimi-Rad, Angila Ataei-
Pirkooh,Elham Kazemi-Rad, Reza Raoofian
Maryam Noshadokht, Amin Yazdani, Sakineh KhamesiJalal Ali Abadi
Marzieh Asadi, Masoud Hamzehkhani, Mohammad Fallah
Marzieh Asadi, Mohammad Fallah, Iiraj Sedighi, Massoud Saidijam, Amir Hossein Maghsood
Marzieh Ashrafmansouri, Bahador Shahriari, Gholamreza Hatam, Samane Abdolahi199
Marzieh Kefayat, Hossein Hamidinejat, Masoud Reza Seifi Abad Shapoori, Parviz Shayan,
Mohammad Mahdi Namavari42
Masomeh Bayani, Narges Kalantari, Salman Ghaffari, Novin Nikbakhsh, Mahmoud Sadegi,
Mostafa Javanian, Hamed Salamatfar, Zeinab Mohseni Afshar
Masomeh Mardani Kataki' Mmehdi Tavalla, Shahnaz Eksear, Mehrdad Sharifi, Sanaz Tavakoli
Masoomeh Ghaderipour, Mostafa Rezaeian, Khadijeh Khanaliha, Maryam Barkhori, Hamed
Mirjalali
Masoud Hamzekhani, Amirhossein Maghsood, Mohammad Matini, Mehrdad Hajilooei,
Mohammad Fallah
Masoud Soosaraei, Abdol Sattar Pagheh, Shirzad Gholami
Masoumeh Ghadam, Asma Askari, Hoseinali Ebrahimzade Mousavi, Poulin Shohreh297
Masoumeh Ghadam, Asma Askari, Poulin Shohreh, Hoseinali Ebrahimzade Mousavi, Mehdi
Bamorovat
Mazaher Gholipour Malekabadi, Zohreh Lasjerdi, Masoud Mozafari, Marzieh Sameni, Vahid
Fallah Omrani, Monochehr Valizadeh, Ali Rostami125
Mehdi Karamian, Mohammad Amin Ghatei, Mina Hemmati181
Mehdi Mohebali, Zahra Kakooei Behnaz Akhoundi, Abbas Rahimi Foroshani
Mehdi Mohtashame Pour, Nader Pestehchian, Ismail Falah, Hosseein Ali Yousefi, Shervin
Ghaffari





Guilan, Iran; 20-22 May 2015
Mehdi Najm , Mahdi Fakhar , Mohammad Shokrzadeh , Seyyedeh Mahboubeh Hosseini ,
Farshideh Habibi , Mehdi Sharif44
Mehdi Najm, Mahdi Fakhar, Bahman Rahimi Esboui, Morad Shaker, Iraj Moubedi248
Mehdi Nateghpour, Afsane Motevali Haghi, Omid Feizi216
Mehdi Nateghpour, Gholamhossein Edrissian, Afsaneh Motevalli Haghi, Leila Farivar,
Eelham Kazemi-Rad41
Mehdi Parsaii, Sedigeh Sarafraz, Mohammad Fatolahzadeh, Hosein Najafzadeh, Nasrin
Gaemmagammi
Mehdi Tavalla, Ebrahim Kazemi
Mehdi Tavalla, Masomeh Mardani247
Mehdi Tavalla, Mohammad Sabaghan, Fateme Asgarian
Mehmet Fatih Aydin
Mehmet Fatih Aydın
Mehmet Fatih Aydin, Ali Sahin209
Mehmet Fatih Aydin, Ferda Sevinc, Mutlu Sevinc
Mehmet Fatih Aydin, Hikmet Kocaman
Mehmet Fatih Aydin, Nazir Dumanli
Mehmet Fatih Aydın, Süleyman Gökmen
Mehmet Fatih Aydin, Suleyman Gokmen, Ali Sahin111
Mehrnush Modaresinejad, Hossien Nahrevanian, Esmat Bagheri Garmarodi, Shohre Khatami,
Fatemeh Sadat Ghasemi
Mehrzad Saraei Sahne Saraee ,Shaghayegh Nozari , Abbas Azadmehr ,Hassan Jahanihashemi ,
Marjan Nassiri-Asl , Mohtaram Adineh ,Farzaneh Javadi-Marand ,Mojtaba Shahnazi321
Mehrzad Saraei, Farzane Javadi-Marand, Abbas Azadmehr Marjan Nassiri-Asl, Shaghayegh
Nozari, Mohtaram Adine Mojtaba Shahnazi, Reza Hajiaghaee
Mehrzad Saraei, Mohtaram Adineh, Abbas Azadmehr, Hassan Jahanihashemi, Marjan Nassiri-
Asl, Shaghayagh Nozari, Farzane Javadi-Marand, Mojteba Shahnazi
Mehrzad Saraei, Saeedeh Shojaee, Yosef Ghaderi, Tahereh Mosavi, Mojtaba Shahnazi,
Mehrzad Saraei, Saeedeh Shojaee, Yosef Ghaderi, Tahereh Mosavi, Mojtaba Shahnazi, Hossein Keshavarz
Mehrzad Saraei, Saeedeh Shojaee, Yosef Ghaderi, Tahereh Mosavi, Mojtaba Shahnazi, Hossein Keshavarz
Mehrzad Saraei, Saeedeh Shojaee, Yosef Ghaderi, Tahereh Mosavi, Mojtaba Shahnazi, Hossein Keshavarz
Mehrzad Saraei, Saeedeh Shojaee, Yosef Ghaderi, Tahereh Mosavi, Mojtaba Shahnazi, Hossein Keshavarz
Mehrzad Saraei, Saeedeh Shojaee, Yosef Ghaderi, Tahereh Mosavi, Mojtaba Shahnazi,Hossein Keshavarz323Mehrzad Saraei, Tahereh Mousavi, Hassan Azhdari-Zarmehri, Benyamin Alimohammadi,Yosef Ghaderi, Marjan Nassiri-Asl, Mojtaba Shahnazi327Mehrzad Saraei, Yosef Ghaderi, Marjan Nassiri-Asl, Tahereh Mousavi, Hassan Jahanihashemi,Mojtaba Shahnazi326
Mehrzad Saraei, Saeedeh Shojaee, Yosef Ghaderi, Tahereh Mosavi, Mojtaba Shahnazi, Hossein Keshavarz323Mehrzad Saraei, Tahereh Mousavi, Hassan Azhdari-Zarmehri, Benyamin Alimohammadi, Yosef Ghaderi, Marjan Nassiri-Asl, Mojtaba Shahnazi327Mehrzad Saraei, Yosef Ghaderi, Marjan Nassiri-Asl, Tahereh Mousavi, Hassan Jahanihashemi, Mojtaba Shahnazi326Meisam Sabour Darbandi, Amin Ahmadi79
Mehrzad Saraei, Saeedeh Shojaee, Yosef Ghaderi, Tahereh Mosavi, Mojtaba Shahnazi, Hossein Keshavarz323Mehrzad Saraei, Tahereh Mousavi, Hassan Azhdari-Zarmehri, Benyamin Alimohammadi, Yosef Ghaderi, Marjan Nassiri-Asl, Mojtaba Shahnazi327Mehrzad Saraei, Yosef Ghaderi, Marjan Nassiri-Asl, Tahereh Mousavi, Hassan Jahanihashemi, Mojtaba Shahnazi326Meisam Sabour Darbandi, Amin Ahmadi79Meisam Sabour Darbandi, Behnam Meshgi, Fatemeh Jalousian282
Mehrzad Saraei, Saeedeh Shojaee, Yosef Ghaderi, Tahereh Mosavi, Mojtaba Shahnazi, Hossein Keshavarz323Mehrzad Saraei, Tahereh Mousavi, Hassan Azhdari-Zarmehri, Benyamin Alimohammadi, Yosef Ghaderi, Marjan Nassiri-Asl, Mojtaba Shahnazi327Mehrzad Saraei, Yosef Ghaderi, Marjan Nassiri-Asl, Tahereh Mousavi, Hassan Jahanihashemi, Mojtaba Shahnazi326Meisam Sabour Darbandi, Amin Ahmadi79Meisam Sabour Darbandi, Behnam Meshgi, Fatemeh Jalousian282Meysam Sharifdini, Hossein Mirhendi, Keyhan Ashrafi, Mostafa Hosseini, Mehdi Mohebali,
Mehrzad Saraei, Saeedeh Shojaee, Yosef Ghaderi, Tahereh Mosavi, Mojtaba Shahnazi, Hossein Keshavarz323Mehrzad Saraei, Tahereh Mousavi, Hassan Azhdari-Zarmehri, Benyamin Alimohammadi, Yosef Ghaderi, Marjan Nassiri-Asl, Mojtaba Shahnazi327Mehrzad Saraei, Yosef Ghaderi, Marjan Nassiri-Asl, Tahereh Mousavi, Hassan Jahanihashemi, Mojtaba Shahnazi326Meisam Sabour Darbandi, Amin Ahmadi79Meisam Sabour Darbandi, Behnam Meshgi, Fatemeh Jalousian282Meysam Sharifdini, Hossein Mirhendi, Keyhan Ashrafi, Mostafa Hosseini, Mehdi Mohebali, Hossein Khodadadi, Eshrat Beigom Kia54
Mehrzad Saraei, Saeedeh Shojaee, Yosef Ghaderi, Tahereh Mosavi, Mojtaba Shahnazi, Hossein Keshavarz323Mehrzad Saraei, Tahereh Mousavi, Hassan Azhdari-Zarmehri, Benyamin Alimohammadi, Yosef Ghaderi, Marjan Nassiri-Asl, Mojtaba Shahnazi327Mehrzad Saraei, Yosef Ghaderi, Marjan Nassiri-Asl, Tahereh Mousavi, Hassan Jahanihashemi, Mojtaba Shahnazi326Meisam Sabour Darbandi, Amin Ahmadi79Meisam Sabour Darbandi, Behnam Meshgi, Fatemeh Jalousian282Meysam Sharifdini, Hossein Mirhendi, Keyhan Ashrafi, Mostafa Hosseini, Mehdi Mohebali, Hossein Khodadadi, Eshrat Beigom Kia54MH Motazedian, N Ghobakhloo, M Ouellette, D Legare, P Leprohon38
Mehrzad Saraei, Saeedeh Shojaee, Yosef Ghaderi, Tahereh Mosavi, Mojtaba Shahnazi,Hossein Keshavarz323Mehrzad Saraei, Tahereh Mousavi, Hassan Azhdari-Zarmehri, Benyamin Alimohammadi,Yosef Ghaderi, Marjan Nassiri-Asl, Mojtaba Shahnazi327Mehrzad Saraei, Yosef Ghaderi, Marjan Nassiri-Asl, Tahereh Mousavi, Hassan Jahanihashemi,Mojtaba Shahnazi326Meisam Sabour Darbandi, Amin Ahmadi79Meisam Sabour Darbandi, Behnam Meshgi, Fatemeh Jalousian282Meysam Sharifdini, Hossein Mirhendi, Keyhan Ashrafi, Mostafa Hosseini, Mehdi Mohebali,Hossein Khodadadi, Eshrat Beigom Kia54MH Motazedian, N Ghobakhloo, M Ouellette, D Legare, P Leprohon38Mina Farazandehmehr, Hooman Rahmati Holasoo, HosseinAli Ebrahim Zadeh Mousavi,
Mehrzad Saraei, Saeedeh Shojaee, Yosef Ghaderi, Tahereh Mosavi, Mojtaba Shahnazi,Hossein Keshavarz323Mehrzad Saraei, Tahereh Mousavi, Hassan Azhdari-Zarmehri, Benyamin Alimohammadi,Yosef Ghaderi, Marjan Nassiri-Asl, Mojtaba Shahnazi327Mehrzad Saraei, Yosef Ghaderi, Marjan Nassiri-Asl, Tahereh Mousavi, Hassan Jahanihashemi,326Mojtaba Shahnazi326Meisam Sabour Darbandi, Amin Ahmadi79Meisam Sabour Darbandi, Behnam Meshgi, Fatemeh Jalousian282Meysam Sharifdini, Hossein Mirhendi, Keyhan Ashrafi, Mostafa Hosseini, Mehdi Mohebali,54Hotazedian, N Ghobakhloo, M Ouellette, D Legare, P Leprohon38Mina Farazandehmehr, Hooman Rahmati Holasoo, HosseinAli Ebrahim Zadeh Mousavi,327Seyyed Saeed Mirzargar, Maryam Alsadat Mirdamadi277
Mehrzad Saraei, Saeedeh Shojaee, Yosef Ghaderi, Tahereh Mosavi, Mojtaba Shahnazi, Hossein Keshavarz323Mehrzad Saraei, Tahereh Mousavi, Hassan Azhdari-Zarmehri, Benyamin Alimohammadi, Yosef Ghaderi, Marjan Nassiri-Asl, Mojtaba Shahnazi327Mehrzad Saraei, Yosef Ghaderi, Marjan Nassiri-Asl, Tahereh Mousavi, Hassan Jahanihashemi, Mojtaba Shahnazi327Mehrzad Saraei, Yosef Ghaderi, Marjan Nassiri-Asl, Tahereh Mousavi, Hassan Jahanihashemi, Mojtaba Shahnazi326Meisam Sabour Darbandi, Amin Ahmadi79Meisam Sabour Darbandi, Behnam Meshgi, Fatemeh Jalousian282Meysam Sharifdini, Hossein Mirhendi, Keyhan Ashrafi, Mostafa Hosseini, Mehdi Mohebali, Hossein Khodadadi, Eshrat Beigom Kia54MH Motazedian, N Ghobakhloo, M Ouellette, D Legare, P Leprohon38Mina Farazandehmehr, Hooman Rahmati Holasoo, HosseinAli Ebrahim Zadeh Mousavi, Seyyed Saeed Mirzargar, Maryam Alsadat Mirdamadi277Mina Hemmati, Mehdi Karamian, Alireza Salehabadi126
Mehrzad Saraei, Saeedeh Shojaee, Yosef Ghaderi, Tahereh Mosavi, Mojtaba Shahnazi,Hossein Keshavarz323Mehrzad Saraei, Tahereh Mousavi, Hassan Azhdari-Zarmehri, Benyamin Alimohammadi,Yosef Ghaderi, Marjan Nassiri-Asl, Mojtaba Shahnazi327Mehrzad Saraei, Yosef Ghaderi, Marjan Nassiri-Asl, Tahereh Mousavi, Hassan Jahanihashemi,326Meisam Sabour Darbandi, Amin Ahmadi79Meisam Sabour Darbandi, Behnam Meshgi, Fatemeh Jalousian282Meysam Sharifdini, Hossein Mirhendi, Keyhan Ashrafi, Mostafa Hosseini, Mehdi Mohebali,54Hotazedian, N Ghobakhloo, M Ouellette, D Legare, P Leprohon38Mina Farazandehmehr, Hooman Rahmati Holasoo, HosseinAli Ebrahim Zadeh Mousavi,277Mina Hemmati, Mehdi Karamian, Alireza Salehabadi126Mitra Salehi, Pejman Bahari, Mohsen Seyed Abadi, Ahmad Mosazade, Amir Adinezade148
Mehrzad Saraei, Saeedeh Shojaee, Yosef Ghaderi, Tahereh Mosavi, Mojtaba Shahnazi,Hossein Keshavarz323Mehrzad Saraei, Tahereh Mousavi, Hassan Azhdari-Zarmehri, Benyamin Alimohammadi,Yosef Ghaderi, Marjan Nassiri-Asl, Mojtaba Shahnazi327Mehrzad Saraei, Yosef Ghaderi, Marjan Nassiri-Asl, Tahereh Mousavi, Hassan Jahanihashemi,326Meisam Sabour Darbandi, Amin Ahmadi79Meisam Sabour Darbandi, Behnam Meshgi, Fatemeh Jalousian282Meysam Sharifdini, Hossein Mirhendi, Keyhan Ashrafi, Mostafa Hosseini, Mehdi Mohebali,54Hotazedian, N Ghobakhloo, M Ouellette, D Legare, P Leprohon38Mina Farazandehmehr, Hooman Rahmati Holasoo, HosseinAli Ebrahim Zadeh Mousavi,277Mina Hemmati, Mehdi Karamian, Alireza Salehabadi126Mitra Salehi, Pejman Bahari, Mohsen Seyed Abadi, Ahmad Mosazade, Amir Adinezade148
Mehrzad Saraei, Saeedeh Shojaee, Yosef Ghaderi, Tahereh Mosavi, Mojtaba Shahnazi,Hossein Keshavarz323Mehrzad Saraei, Tahereh Mousavi, Hassan Azhdari-Zarmehri, Benyamin Alimohammadi,Yosef Ghaderi, Marjan Nassiri-Asl, Mojtaba Shahnazi327Mehrzad Saraei, Yosef Ghaderi, Marjan Nassiri-Asl, Tahereh Mousavi, Hassan Jahanihashemi,Mojtaba Shahnazi326Meisam Sabour Darbandi, Amin Ahmadi79Meisam Sabour Darbandi, Behnam Meshgi, Fatemeh Jalousian282Meysam Sharifdini, Hossein Mirhendi, Keyhan Ashrafi, Mostafa Hosseini, Mehdi Mohebali,Hossein Khodadadi, Eshrat Beigom Kia54MH Motazedian, N Ghobakhloo, M Ouellette, D Legare, P Leprohon38Mina Farazandehmehr, Hooman Rahmati Holasoo, HosseinAli Ebrahim Zadeh Mousavi,277Mina Hemmati, Mehdi Karamian, Alireza Salehabadi126Mitra Salehi, Pejman Bahari, Mohsen Seyed Abadi, Amir Adinezade, Amir Adinezade148Mitra Salehi, Pejman Bahari, Mohsen Seyed abadi, Amir Adinezade, Amir Adinezade148
Mehrzad Saraei, Saeedeh Shojaee, Yosef Ghaderi, Tahereh Mosavi, Mojtaba Shahnazi,Hossein Keshavarz323Mehrzad Saraei, Tahereh Mousavi, Hassan Azhdari-Zarmehri, Benyamin Alimohammadi,Yosef Ghaderi, Marjan Nassiri-Asl, Mojtaba Shahnazi327Mehrzad Saraei, Yosef Ghaderi, Marjan Nassiri-Asl, Tahereh Mousavi, Hassan Jahanihashemi,326Meisam Sabour Darbandi, Amin Ahmadi79Meisam Sabour Darbandi, Behnam Meshgi, Fatemeh Jalousian282Meysam Sharifdini, Hossein Mirhendi, Keyhan Ashrafi, Mostafa Hosseini, Mehdi Mohebali,54Hotazedian, N Ghobakhloo, M Ouellette, D Legare, P Leprohon38Mina Farazandehmehr, Hooman Rahmati Holasoo, HosseinAli Ebrahim Zadeh Mousavi,277Mina Hemmati, Mehdi Karamian, Alireza Salehabadi126Mitra Salehi, Pejman Bahari, Mohsen Seyed Abadi, Ahmad Mosazade, Amir Adinezade148





Guilan, Iran; 20-22 May 2015	
Mmohammad Reza Karimazar, Mohsen Najjari, Gholamali Karimi94	ŀ
Moazmah Andishmand, Naser, Zia Ali, Mohammd Ahmadinejad, Lila Malakeian	
Mohaddeseh Abouhosseini, Mohammad Reza Youssefi	1
Mohaddeseh Khaksar, Narges Kalantari, Salman Ghaffari, Hosein Abbaspour, Seyed-Mehdi	
Hamidekish, Taraneh Ghaffari	;
Mohamad Bagher Khadem Erfan, Ghasem Zamini, Ashkan Faridi, Fares Bahrami73	;
Mohamad Bagher Khadem Erfan, Ghasem Zamini, Ashkan Faridi, Hamid Salehinia	;
Mohamad Hasan Kohansal, Abbasali Nourian, Saeed Bafandeh121	
Mohamad Javad Ranjbar, Bahador Shariari, Gholamreza Mowlavi, Zienab Seifullahi76	
Mohammad Ali Mohammadi, Balal Sadeghi, Majid Fasihi Harandi138	;
Mohammad Amin Ghatee, Fatemeh Koreshnia, Ali Akbar Haghdoost, Zahra Kanannejad,	
Zafar Parisae, Abdol Ali Moshfea173	;
Mohammad Bagher Ahoo, Seyed Hossein Hosseini' Fatemeh Jalousian, Zahra Jahani Chegeni,	,
Amin Bakhshani	
Mohammad Bagher RoknI, Bahram Kazemi, Mojgan Aryaeipour, Soheila Rouhani, Mojgan	
Bandehpour, Hadi Mirahmadi96	j
Mohammad Barati , Mehdi Mohebali , Mohammad Hossein Alimohammadian , Ali	
khamesipour, Hossein Keshavarz, Behnaz Akhoundi, Zabiholah Zarei35	,
Mohammad Ebrahimipour, Aziz Kassani, Hedayat Abbastabar104	
Mohammad Ebrahimipour, Mohsen Najjari, Aziz Kassani122	2
Mohammad Ebrahimipour	
Mohammad Fallah, Amir Hossein Maghsood, Rasool Jafari156	j
Mohammad Fallah, Morteza Shamsi, Afra Khosravi, Amirhossin Maghsood, Alimohammad	
Bahrami, Reza Hoshmandfar139	
Mohammad Fallah, Saeed Bajalan, Amirhossein Maghsood, Alireza Zamani	ŀ
Mohammad Fatollahzadeh, Majid khan Mohammadi, Ahad Bazmani, Nasrin Mirsamadi,	
Rasool Jafari, Mehdi Mohabali, Taher Nemati, Esmail Fallah	2
Mohammad Fatollahzadeh, Sedige Sarafraz, Nader Ghabouli Mehrabani, Vahid Rahnama,	
Taher Nemati, Nasrin Ghaem Maghami, Mohammad Reza Khalili	
Mohammad H Motazedian, Qasem Asgari, Belal Armand	
Mohammad Hossein Feiz Haddad, Iman Khodkar, Rezvan Feiz Haddad	
Mohammad Hossein Motazedian	
Mohammad Hossein Radfar, Sogand Lahooti, Ali Pezeshkpour	
Mohammad Jafari Modrek, Mohammad Mousavi, Ramin Saravani	
Mohammad Jalalvand, Nasrin Moghadar, Meisam Sabour darbandi, Elham Malmir	,
Mohammad Javad Abbaszadeh Aafshar, Mehdi Mohebali Mohebali, Iraj Sharifi, Behnaz	
Akhoundi, Mohammad Reza Aflatoonian, Mohammad Saleh Bahreini, Hossein	1
Mahmoudvand	
Mohammad Matini	
Mohammad Matini, Khadijeh Rahmati, Mohammad Fallah, Tayebeh Shamsi-Ehsan	ł
Mohammad Matini, Mostafa Rezaeian, Mahdi Mohebali, Amir Hossein Maghsood, Mohammad fallah, Soghra Pabiaa	,
Mohammad fallah, Soghra Rabiee	
Mohammad Matini, Tayebeh Shamsi-Ehsan, Amir Hossein Maghsood, Khadijeh Rahmati.237	
Mohammad Motazedian	
Mohammad Mousavi, Ramin Saravani, Mohammad Jafari Modrek	
Mohammad Mousavi, Sina Sekandarpour	





Guilan, Iran; 20-22 May 2015
Mohammad Rahnama, Javad Khedri, Marie Sedaqati Mokhtari
Mohammad Rahnama, Javad Khedri, Nazanin Ahmadzade263
Mohammad Rayani, Gholam Reza Hatam, Iraj Mohammadpur, Arghavan Vafafar106
Mohammad Rayani, Gholam Reza Hatam, Ngah Zasmy Unyah, Wan Omar Abdullah,
Rukman Awang Hamat
Mohammad Reza Chalchi Nosrati, Ali Eslami
Mohammad Reza Mahmoudi, Bahram Kazemi, Ali Haghighi, Panagiotis Karanis
Mohammad Reza Mahmoudi, Bahram Kazemi, Asad Mirzaei, Anita Mohammadiha,
Panagiotis Karanis
Mohammad Reza Mahmoudi, Fariba Berenji, Abdolmajid Fata, Mohammad Javad
Najafzadeh, Amin Asadian
Mohammad Reza Nilforoushan, Ali Marashi <sup>,</sup> Ali Heidari, Mashaallah Rezaei, Adel Maqsoudi
Mohammad Reza nilforoushan, Vajihoalsadat Davoudi, Faeze Ghazvini
Mohammad Reza Shiee , Zabihollah Zarei , Behnaz Akhoundi , Soudabeh Heidari ,
Mohammad Taghi Satvat , Sorour Charehdar ,Zahra Kakooei , Mohammad Reza Abai , Mehdi
Mohebali
Mohammad Reza Shiee, Mehdi Mohebali, Homa Hajjaran, Rezvan Pourmoayied, Batool
Abani, Jahangir Sarafrazi
Mohammad Reza Vafaei, Mohammad Ali Mohaghegh, Mohsen Ghomashlooyan, Mojtaba
Azimi Resketi, Hamed Kalani, Farzaneh Mirzaie, Mohammad Falahati, Yaghob Azadi158
Mohammad Reza Youssefi
Mohammad Reza Youssefi, Alireza Barimani, Amanta Fard
Mohammad Reza Youssefi, Mohammad Taghi252
Mohammad Sadaghian, Ali Hayatrouhi, S. Mostafa Niknezhad
Mohammad Sadaghian, Sina Moghaddam
Mohammad Sadaghian, Tarlan Farahvash, Ali Mehrshad100
Mohammad Taghi Ahady, Behroz Ahmadi, Naghmeh Ahadi, Leila Asadi233
Mohammad Taghi Rahimi, Afsane Amooei, Ehsan Ahmadpour, Bahman Rahimi Esboei, Adel
Spotin, Mohammad Hasan Kohansal Koshki, Ahad Alizadeh, Soheila Honary, Hamed
Barabadi, Milad Ali Mohammadi, Azadeh Mizani119
Mohammad Taghi Rahimi, Mohammad Mehdi Darvishi, Parviz Shayan, Mohammad Reza
Youssefi, Emad Changizi
Mohammad Taghi Rahimi, Shahabeddin Sarvi, Ahmad Daryani, Mahdi Sharif, Davood Azami,
Zahra Marhaba, Azadeh Mizani
Mohammad Zibaei, Seyed Mahmoud Sadjjadi, Sharif Maraghi
Mohammad Zibaie, Kourosh Cheraghipour
Mohammadraeza Mahmudi, Sseyed Hossein Mojtabaee, Nahid Bidari, Behnaz Rahmati154
Mohammadreza Foroutani
Mohammadreza Foroutani
Mohammadreza Foroutani
Mohammadreza Foroutani, Seedhannan Kashfi
Mohammadreza Jabbari, Akbar Hossennejad, Mahdi Fakhar, Shirzad Gholami, Eissa
Soleymani
Mohammadreza Karimazar, Ali Ranbarpoor, Mehdi Soleimanfallah, Asghar Soltani,
Mehrnoosh Salami, Zabihollah Fallahnezhad





Guilan, Iran; 20-22 May 2015
Mohammadreza Karimazar, Mehdi Soleimanfallah, Zahra Dadashi, Asghar Soltani, Iraj
Kohshekaf, Mehrnoosh Salami, Behzad Abedini
Mohammadreza Karimazar, Mehdi Soleimanfallah, Zahra Dadashi, Asghar Soltani, Iraj
Kohshekaf, Shiva Asgharian, Zabihollah Fallahnezhad
Mohammadreza mahmoudi, Nastaran Hoseinshiroudi, Yasaman Vahedi, Behnaz Rahmati65
Mohammadreza Mahmoudi, Yasaman Vahedi, Nastaran Hoseinshiroudi, Behnaz Rahmati64
Mohsen Arbabi, Majid Pirestani, Mahdi Delavari, Hossein Hooshyar, Amir Abdoli, Shahab
Sarvi
Mohsen Arbabi, Nader Esmaeli, Karim Parastooei101
Mohsen Arbabi, Zohreh Fakhrieh, Mahdi Delavari
Mohsen Ghomashlooyan, Mohammad Ali Mohaghegh, Mohammad Falahati, Rasool Jafari,
Farzaneh Mirzaie, Hamed Kalani, Ghodratollah Salehi Sangani, Mehdi Azami
Mohsen Ghomashlooyan, Mohammad Ali Mohaghegh, Mojtaba Azimi Resketi, Reza
Mohammadimanesh, Farzaneh Mirzaie, Mehdi Azami, Mohammad Falahati, Hossein Hejazi
Mohsen Najjari
Mohsen Najjari
Mojgan Aryaeipour, Eshrat Beigom Kia, Zahra Heidari, Mohammad Bagher Rokni99
Mojtaba Maghateli, Ghilda Eslami, Morteza Mohamadzadeh, Sajjad Rahimi, Vahid Ajmiyan,
Javad Namrudi, Azita Eshratkhah
Mojtaba Shahnazi, Abbas Azadmehr, Robabeh Latifi, Reza Hajiaghaee, Sahar Mosalla,
Mehrzad Saraei, Mahmood Alipour142
Mojtaba Shahnazi, Abbas Azadmehr, Robabeh Latifi, Reza Hajiaghaee, Sahar Mosalla,
Mehrzad Saraei, Mahmood Alipour142
Mojtaba Shahnazi, Delaram Nahid, Tayebeh Yousephzad, Mehrzad Saraei, Mahmood Alipour,
Abbas Azadmehr
Moloud Nahvi, Saeedeh Shojaee, Mehdi Mohebali, Hossein Keshavarz, Mahboobeh Salimi 316
Moradali Fouladvand, Afshin Barazesh, Soleiman Khorami, Rahim Tahmasebi71
Moradali Fouladvand, Soleiman Khorami, Jamal Fallahi, Rahim Tahmasebi285
Morteza Shamsi, Abdolhossin Dalimi, Afra Khosravi, Fatemeh Ghafarifar, Javid Sadraie144
Morteza Zaim, Ahmad Raeisi
Mosenh Arbabi, Zohre Fakhrie-Kashan , Mahdi Delavari , Mohsen Taghi-Zadeh , Hossein
Hooshyar
Mostafa Zahed Tehrani, Seyed Hossein Hejazi, Zzahra Bamzadeh
Movahedi
Nader Pestechian, Akram Sepahvand, Reza Pahlavan Gharebaba, Ahmad Hosseini Safa,
Hossein Ali Yousefi, Syedeh Fatemeh Moosavi, Reza Mohammadi Manesh107
Nader Pestechian, Hamed Kalani, Roghiyeh Faridnia, Hosein-Ali Yousefi
Nader Pestehchian, Hossein Ali Yousefi, Ahmad Hosseini Safa
Nader Pestehchian, Mohamadreza Etemadifar, Hosinali Yousefi, Maryam Chiani, Neda
Aslani
Nahid Jalallou, Farzad Nursabaghi, Mahmood Abedinzadeh
• •
Nahid Maspi, Fatemeh Ghaffarifar, Zohreh Sharifi, Abdolhossein Dalimi, Vahid Nasiri170 Nahid Maspi, Zahreh Sharifi, Abdolhossein Dalimi, Vahid Nasiri Estemeh Chaffarifar
Nahid Maspi, Zohreh Sharifi, Abdolhossein Dalimi, Vahid Nasiri, Fatemeh Ghaffarifar172 Nahidah Mashari Zain Abad, Abbas Shabbari, Farmacil Fallah, Chadratallah Salahi, Bayman
Nahideh Mazhari Zein Abad, Abbas Shahbazi, Esmaeil Fallah, Ghodratollah Salehi, Peyman
Heydarian127





Guilan, Iran; 20-22 May 2015
Narges Kalantari, Salman Ghaffari, Masomeh Bayani, Mohadeseh Khaksar, Taraneh Ghaffari
Narmin Najafzadeh, Parviz Parvizi, Roozbeh Taslimian
Naser Nazari, Sohylla Rahmani
Nashmin Mohemi, Mohammad Fallah, Mohammad Moradi, Ali Reza Khalilian, Amir Hosein
Maghsoud
Motavalian, Ahmad Reza Meamar
Nasrin Mirsamadi, Saber Alizadeh, Mahdi Mohammadzadeh, Jafar Sadegh Tabrizi, Yasaman
Ghanei Mohammadi
Nasrin Mirsamadi, Saber Alizadeh, Mahdi Mohammadzadeh, Jafar Sadegh Tabrizi, Yasaman
Ghanei Mohammadi
Nasrollah Vahedi Nouri
Nasrollah Vahedi Nouri, Mahmud Golchin, Hussein Furoghi, Hamid Aghaganzaheh112
Nasrollah Vahedi Nouri, Majid Gholampour, Zhila Torabizadeh, Hossin Froghi, Mahmoud
Golchin
Nasser Hajipour, Mousa Tavassoli, Hossein Tayefi, Rohollah Motafaker, Ali Ghorashi
Nateghpour M, Motevalli Haghi A, Farivar L, Hajialilo E, Bozorgomid A
Nazanin Fallah, Khadijeh Rahmati, Mohammad Fallah
Nazir Dumanlı, Kürşat Altay, Mehmet Fatih Aydın
Nona Moradpour, Hassan Borji Gholamreza Razmi, Mohsen Maleki, Hossien Kazemi291
Nona Moradpour, Jamshid Darvish
Nooshin Hashemi, Mehran Bahadoran Bagh Badorani, Mitra Hashemi, Seyed Hossein Hejazi
Omid Ghashghaei, Javad Khedri, Ali Afsharimoghaddam, Forozan Jahangiri226
Panagiotis Karanis
Parvin Ramezani, Rreza Shahri Moghadam, Hossein Hejazi
Payman Ziapour, Sadegh Kheiri, Fatemeh Asgarian, Mahmoud Fazeli-dinan, Reza Ali
Mohammadpour, Fariborz Yazdi, Hasan Nikookar, Mohammad Sarafrazi, Ahmadali Enayati
Pedram HaddadMolayan, Mansour Mayahi, Hossein Hamidinejat, Hedieh Jafari, Leyla
Derakhshan, Omid Hosseininejad
Peter Deplazes
Peyman Ghaemi, Nasser Hoghooghi-Rad, Parviz Shayan, Brigitte Eckert
Pooya Ghasemi Nejad Almani, Iraj Sharifi, Bahram Kazemi, Zahra Babaei, Mojgan
Bandehpour, Samira Salari, Ebrahim Saedi Dezaki, Farideh Tohidi
Rabeeh Tabaripour, Abbas Alizadeh, Mahdi Fakhar, Mohammad Reza Youssefi, Mahdi Sharif
Rahimi
Razieh Ayati, Hhosein Rezvan, Ali Sadeghi Nasab, Bahram Dadmehr
Razieh Tavakoli Oliaee, Hossein Mahmoudvand, Iraj Sharifi, Monireh Sezavar Rahmat186 Reyhaneh Haghighi, Mehdi Golchin, Hamid Daneshvar, Zahra Mahmoodi, Hossin Kamiabi
Reza Ghasemikhah, Mohammad Alimoradi
Reza Kalantari, Javid Sadraei, Majid Pirestani, Mahsa Esmaeili Fallah
NCZa Natahlah, javiu Saulaci, Majiu Filestani, Mansa Esinacin Fanan





Guitan, Iran; 20-22 May 2015
Reza Mohammadi-Manesh, Abbas Ali Eskandarian, Maryam Niyyati, Hosein Ali Yousefi,
Akram Sepahvand, Mohammad Ali Mohaghegh68
Reza Nabavi, Mehdi Rasekh, Ali Sarani
Reza Nabavi, Mohammadreza Hajinezhad, Abbas Jamshidian
Reza Rezaei, Shahram Seyyedy, peyman mikaili, Shahram Khademvatan, Khosrow Hazrati
Tappeh
Reza Rostamipour, Mohammad Zibaei, Hassan Nayebzadeh
Reza Saberi, Alireza Latifi, Samira Shahdost, Maryam Niyyati67
Reza Shafiei, Bahador Sarkari, Seyed Mahmoud Sadjjadi
Reza Shafiei, Bahador Sarkari, Seyed Mahmoud Sadjjadi97
Reza Shafiei, Mahdi Mohebali, Behnaz Akhondi, Meysam Sharifdini, Kurosh Kalantar,
Abdolmajid Fata, Bibi Razieh Hosseini Farash, Mehrdad Ghasemian, Maryam Gholami 190
Reza Soltani
Reza Soltani
Reza Soltani, Ehsan Zayerzadeh
Reza Soltani, Saba Belgheisi
Roghayeh Norouzi, Abdolhossein Dalimi Asl, Mahdi Forouzandeh, Fatemeh Ghaffarifar 309
Roghayeh Norouzi, Abdolhossein Dalimi Asl, Mahdi Forouzandeh, Fatemeh Ghaffarifar310
Roghiyeh Faridnia, Nader Pestechian, Hamed Kalani
Roghiyeh Faridnia, Sepideh Tolouei, Hosein Khanahmad-Shahreza, Hamed Kalani, Hosein
Yousefi-Darani
Roh Allah Abadian, Farhang Babamahmoodi, Shirzad Gholami, Eissa Soleymani284
Roohollah Fattahi, Elahe Ebrahimzade, Mohammad Bagher Ahoo
Roya Khalili, Elham Razmjou, Zahra Pirasteh, Lameh Akhlaghi, Zahra Eslami Rad, Maziyar
Moradi-Lakeh, Ahmad Reza Meamar
S.Hosein Hoseini, Fatemeh Jalousian, Mehdi Borhanizarandy, Reza Mirzaei, Fazeleh Etebar
Saber Olfaty Harsini, Hamidreza Shokrani, Hassan Naiebzadeh
Saber Olfaty Harsini, Hamidreza Shokrani, Moslem Safari, Amir Mansouri253
Saeed Samaei Nasab, Nona Moradpour
Saeedeh Shamsadini Lori, Mohammadali Mohammadi, Seyedreza Mirbadie, Saeid
Nasibidavarani, Balal Sadeghi, Sima Rostami, Majid Fasihiharandi122
Saeedeh Shojaee, Mahboobeh Salimi, Hossein Keshavarz, Mehdi Mohebali
Saeid Fathi, S.Hosein Hoseini, Fatemeh Jalousian, Samaneh Adhaie144
Saeid Nasibi Davarani, Abdoreza Salahi-Moghaddam, Naser Zia-Ali,Mohammad Ali
Mohammadi, Elham Akhlaghi, Majid Fasihi Harandi96
Saeideh Farajzadeh, Maryam Hakimi Parizi, Amireh Heshmatkhah, Ali Akbar Haghdoost,
Abbas \Pardakhty, Mahshid Mostafavi178
Sahar Ebrahimi, Ali Bordbar, Parviz Parvizi, Adel Spotin
Saideh Farajzadeh, Saman Mohammadi, Mahshid Mostafavi, Iraj Esfandiarpour, Ali Akbar
Haghdoost, Maryam Hakimi
Saman Hosseinkhani
Samaneh Abdolahi Khabisi, Bahador Shahriari
Samira Dodangeh, Maryam Niyati, Ali Haghighi, Mohammad Kamalinejad, Farideh Hezari,
Eznolah Azargashb
Sanaz Jafarpoorazami, Saeedeh Shojaee, Mahboobeh Salimi





Guitan, Iran; 20-22 May 2015
Sanaz Taghizadeh Eghtedar, Abbas Shahbazi, Mahmood Mahami, Asghar Salehpour, Helen
Hooshdeghati Sedigheh Sarafraz, Ali Shariatzadeh
Sania karami, Farzaneh Biglarnia, Kavoos Solhjoo, Mohammad Hassan davami, Belal
Armand, Hassan Rezanezhad
Sania Karami, Farzaneh Biglarnia, Kavoos Solhjoo, Mohammad Hassan davami, Belal
Armand, Hassan Rezanezhad
Santiago Mas-Coma
Santiago Mas-Coma
Sara Larki, Mohammad Moazeni, Ahmad Oryan, Mohammadjamal Saharkhiz
Sara Larki, Mohammad Moazeni, Mohammadjamal Saharkhiz, Ahmad Oryan, Maryam Ansari
Lari, Amir Mootabi Alavi
Sara V. Brant, Eric S. Loker
Sattar Pagheh, .Mmahdi Fakhar, Fatemeh Mesgarian, Vahid danesh, Masood Soosaraie,
Hossein Pazoki Ghohe
Sedigeh Sarafraz, Nasrin Gaemmaghami, Kazem Ghahramanzadeh, Mohamadreza Khalili,
Rahman Radmehr, Mohamad Fathalahzaeh, Vahid Rahnema, Mahdi Parsaei
Seedhannan Kashfi, Mohammadreza Foroutani
Seif Ali Mahdavi , Narges Afrakhteh , Sahar Garroosian , Reyhaneh Mirnejad , Haniye Ahmadi
Shahraj, Mahsa Eshkevar Vakili, Ali Norbakhsh, Mohammadtaghi Rahimi, Mahmood
Mosazadeh
Seif Ali Mahdavi
Seif Ali Mahdavi
Seif Ali Mahdavi, Ali Charkameh, Jalil shojaei, Azita Dalili
Seif Ali Mahdavi, Fahimeh Alinejad, Navisa Sadat Seyedghasemi
Seif Ali Mahdavi, Jalil Shojaei, Azita Dalili, Gholamreza Ghorbani, Abolhassan Mansoorian, Ali
Charkameh
Seif Ali Mahdavi, Jalil Shojaei, Hormoz Kianian, Ali Charkameh, Abolhassan Mansoorian,
Ggholamreza Ghorbani, Asghar Nezammahalleh
Setareh Askari, Mehdi Nateghpour, Afsaneh Motevalli Haghi
Seyed Mahmoud Sadjjadi, Ali Noormohammadi, Tahereh Mohammadzadeh, Abdolmajid Fata
Seyed Mousa Motavalli Haghi, Mahdi fakhar, Abdolsatar paghe, Mitra Sharbatkhor, Shirzad
Gholami, Mohammad Reza Yussefi
Seyed Mousa Motavalli Haghi, Mahdi fakhar, Abdolsatar paghe, Mitra Sharbatkhori, Shirzad
gholami
Seyed Sajjad Hasheminasab
Seyed Sajjad Hasheminasab, Hadi Mohammadi Talvar, Hossein Maghsood
Seyedeh Saryam Shrafi
Seyedeh Sekineh Khamesi, Maryam Noshadokht, Jalal Aliabadi
Seyedreza Mirbadie, Mohammadali Mohammadi, Saeedeh Shamsadini Lori, Ebrahim Saedi
Dezaki, Hosain Kkamyabi, Majid Fasihiharandi
Seyyed Ali Shariatzadeh ,Abbas shahbazi , Esmaeil fallah , Shirzad Gholami ,Adel Spotin ,
Fattaneh Montazery, Abbas Najafinasab, Sanaz Taghizadeeghtedar
Seyyede Nassim Tabatabaee, Hasan Borji, Abulghasem Naghibi
Shadi Salamroodi, Safa Norouzi, Bijan Majidi Shad
Shahab Shams, khadijeh khanaliha, Tahereh Mohammadzadeh, Saman Amiri Emami,
Mohammad Hossein Marhamatizadeh





Guilan, Iran; 20-22 May 2015
Shahabeddin Sarvi, Ahmad Daryani, Mehdi Sharif, Ehsan Ahmad Pour, Azar Shokri,
Mohammad Taghi Rahimi, Mohsen Aarabi , Azadeh Mizani
Shahabeddin Sarvi, Ahmad Daryani, Mehdi Sharif, Mohammad Taghi Rahimi, Davood
Aezami, Zahra Marhaba Ehsan Ahmadpour, Azadeh Mizani, Azar Shokri136
Shahla Jahan Bin, Farshad Hoseyni, Urang Ilami, Abdolali Moshfe, Bahador Shahryari <sup>,</sup> Saeed
Jokar
Shahnaz Shirbazou, Hedayat Sahraei, Vahdat Hajihoseinloo, Hasan Ghoshoni
Shahram Khademvatan, Monir Dudi, Sadigheh Albalhit
Shahrokh Shirazi, Rasool Madani, Nasser Hoghooghi-Rad, Fariba Golchinfar, Tara Emami,
Arash
Shamim Toopchi, Mohammad Hossein Motazedian, Ghasem Asqari, Mehdi Ghazanfari,
Shahrbanoo Naderi, Mehdi Mohebali, Behnaz Akhoondi
Sharif Maraghi, Mehdi Tavalla, Mohammad Hossein Feiz Haddad, Firooz Shahrivar
Kolvanagh
Sharif Maraghi, Mohammad Hossein Feiz Hadda, Mehdi Tavalla, Zeinab Laali
Sharif Marraghi, Firooz Shahrivar Kolvanagh, Mehdi Tavalla, Somaye Azimi
Shima Danesh Pour, Hossein Yousofi Darani
Shima Rezaii Afarmajani, Mohammad Taghi Ahady, Hashem Yagoobi
Shirzad Fallahi, Ali Rostami, Bahram Delfan, Yadollah Pournia, Marzieh Rashidipour101
Shirzad Fallahi, Ali Rostami, Farzad Ebrahimzadeh, Yadollah Pournia
Shirzad Fallahi, Zahra Arabmazar, Mehrdad Ghasemian, Seyyed Javad Seyyedtabaei, Bahram
Kazemi
Shohreh Aminpour, Mehdi Tavalla
Sima Rasti, Baharak Ghorbanzadeh, Farnaz Kheirandish, Gholamabbas Mousavi, Ahmad
Pirozmand, Hossien Hooshyar, Bathol Abani170
Sima Rasti, Fatemeh Sadat Ghasemi, Ahmad Piroozmand, Mojgan Bandehpour, Bahram
Kazemi, Seyed Gholam Abbas Mousavi
Soheila Molaie, Abdolhosein Dalimi Asl, Mehdi Mohebali, Zabih Zarei, Behnaz Akhoundi .184
Soheila Molaie, Mehdi Mohebali
Somayeh Mirzavand, GholamReza Hatam, MahmoodReza Moein, MohammadMehdi
Zarshenas
Somayeh Molae Saghin, Rreza Kheyrandish, Mohammad Hosein Radfar, Maryam Hedayati,
Amin Masnavi Poor, Zahra Asadi
Soudabeh Etemadi , Soudabeh Heidari , Zahra Heidari , Mohammad Momeni , Parvaneh
Sarani Ali Abadi , Bahareh Kamranrashani , Eshrat Beigom Kia132
Soudabeh Heidari, Soudabeh Etemadi, Zahra Heidari, Bahar Kamranrashani, Mohammad
Momeni, Parvaneh Sarani Ali Abadi, Eshrat Beigom kia
Sshahnaz Shirbazou, Ali Khamesi Pour, Nahid Masbi, Fatemeh Ghaffari Far, Sara Damghani
Sung-Tae Hong
Taher Elmi, Hajar Ziaie, Bahman Rahimi, Zahra Geraili, Shirzad Gholami
Taher Nemati, Mohammad Fatollahzadeh, Nader Ghabouli Mehrabani, Esmail Fallah, Majid
Khanmohammadi, Ahad Bazmani, Mohammad Hasan Kohansal Koshki, Nasser Hajipour.189
Tahereh Zavari, Sakineh Mirzaei, Mohsen Rezaeian, Nahid Zainodini, Mohammad Zare-
Bidaki
Tayyebeh Karimi, Iraj Sharifi, Mohammad Reza Aflatoonian, Zahra Babaei, Amireh Heshmat
khah, Mino Mashaykhy, Behnaz Aflatoonian, Mohammad Ali Mohammadi





Guilan, Iran; 20-22 May 2015
Ülgen Zeki Ok
Vafa Saber, Maryam Nourozi, Seyyed Javad Seyyed Tabaei, Hooman Hanifehpour, Ghazaleh
Amjadi, Mohammad Reza Behravan
Vahdane Mohamadi Pour, Maryam Mazallahi177
Vahdat Haji hoseinlou, Shahnaz Shirbazou, Hedayat Sahraei150
Vahid Ajamein, Gilda Eslami
Vahid Fallah Omrani, Farnaz Kheirandish, Soheila Rouhani, Bahram Kazemi, Seyed Javad
Seyed Tabaei, Mojgan Bandehpour129
Vahid Rahnamay Hayati Hagh, Mahmoud Mahami Oskouei, Ahad Bazmani, Abolfazl
Miahipour, Nasrin Mirsamadi, Mina Bafandeh
Vahid Tayefi Ardabili, Mohammad Taghi Ahady, Reza Didevar
Yaghoobi-Ershadi
Yan J, HS Kim, JH Kim, CH Yoon, EH Hwang, H Kang, SY An, KJ Kim, ST Hong
Yasaman Alijany, Maryam Ghavami, Gilda Eslami
Yasaman Norouzi Larki, Bahador Shahriari, Qasem Asgari, Samaneh Abdolahi Khabisi329
Yavar Rassi
Zahabiun
Zahra Arab-Mazar, Seyyed Javad Seyyed-Tabaei, Ameneh Koochaki, Shirzad Fallahi
Zahra Asadi, Reza Kheyrandish, Maryam Hedayati, Mohammad Hosein Radfar, Somayeh
Mollae Saghin
Zahra Barzin, Seyyed Mahmoud Sadjjadi, Mohammad Reza Panjehshahin
Zahra Chizari, Hossein Hejazi, Atefe Salehi, Mohammad Ali Mohaghegh, Mohsen
Ghomashlooyan, Reza Mohammadi manesh
Zahra Eslamirad, Homa Soleimani, Reza Hajihossein
Zahra Eslamirad, Reza Hajihossein, Behzad Ghorbanzadeh, Amir Almasi Hashianee
Zahra Gharibi, Mohammad Fallah, Amir Hossein Maghsood, Masoud Saeedi Jam, Farahnaz
Dadras, Gholam Reza Hatam
Zahra Heidari, Iraj Mobedi, Seyed Hossein Mirhendi, Fattaneh Mikaeili, Zabihallah Zarei,
Eshrat Beigom
Zahra Heidari, Iraj Mobedi, Zabihallah Zarei, Meysam Sharifdini, Eshrat Beigom Kia
Zahra Jafarpur, Ismail Fallah, Mahmood Mahami, Ali Haghighi, Maryam Niyati, Afsaneh
Dolatkhah, Aram Khezri
Zahra Mahmoodi, Hamid Daneshvar, Reyhaneh Haghighy, Hossein Kamyabi
Zahra Nasiri, Davood Keshvarzi
Zahra Rashno, Abbas Hatami, Siamak Amiri
Zahra Rashno, Abbas Hatami, Siamak Amiri, Firooz Beiranvand
Zahra Safaee, Zarrintaj Valadkhani, Mojtaba Sohrabi
Zahra Zarei, Daroush Yarahmadi, Mansour Halimi185
Zarrintaj Valadkhani, Abolfazl Mirzadeh, Asieh Yoosefy25
Zeinab Abiri, Asma Askari, Hamed Ganjali, Mahla Ganjali216
Zeinab Seifullahi, Bahador shahriari, Mohammad Hossein Motazedian, Ghasem Asghari,
Mohammad Javad Ranjbar
Zeynab Farrokhy Kary Bozorg, Aliehsan Heidari, Vahideh Moein Vaziri, Ali Haghighi, Javad
Seyyed Tabaei1, Alireza Abadi, Mahboobeh Salimi218
Zivar Sadeghi Dehkordi, Aarjang Partoanadazanpor, Noshin Abdolmaleki
Zivar Sadeghi Dehkordi, Ali Mahmoodi91





163
163
342
339





Proceedings of 2nd International and 9th National Congress of Parasitology and Parasitic Diseases of Iran (NICOPA 9)

Guilan, Iran; 20-22 May 2015

#### FROM MOSQUITO CONTROL TO VECTOR CON-TROL IN SOUTHERN EUROPE: INNOVATIVE STRATEGIES TO TACKLE THE ISSUE

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This contribution aims to give an overview of mosquito control; mosquitoes have recently become vectors of VBD. The presentation provides state of the art information on the issue. In southern Europe including northern Italy, vast areas of wetlands exist during most of the year as a result of rice cultivation. It is estimated that nearly 240,000 ha of irrigated rice fields prevail annually in the provinces of Alessandria and Pavia, Italy. These rice fields are considered as the largest European habitat of mosquitoes providing ideal breeding places for several mosquito species, particularly several generations of Ochlerotatus caspius and Culex spp., and Anopheles maculipennis each year. The dispersal of these species, some of which are newly recognised vectors of VBD, is new issues to tackle. Even malaria as reported by ECDC, is mainly a travel medicine issue because a large number of reported cases are imported; recent investigation considers nonetheless, 'airport malaria' is sometimes reported in relation to the inadvertent transport of infected mosquitoes from endemic areas. Recent investigation based on molecular identification confirm Anopheles maculipennis are An. Messae, by, An, maculipennis, An. sacharovi, An. messae and An. Atroparvus Aedes albopictus, first detected in Italy in 1990, in 2007, has been incriminated as the main vector of the first European outbreak of Chikungunya virus (CHIKV) in northern Italy and information on high vector competence of this pestiferous makes these above mentioned Culicidae ofsocio economic importancealso responsible forarising public health concern for VBD transmission. Despite the progressive acquisition of knowledge and relatively enormous resources designated globally for tackling human health problems, the potential public health concerns associated with vectors and those related to vector capacity are generally ignored in the developing European countries.

Keywords: vector control, Ochlerotatus caspius, Anopheles maculipennis, Aedes albopictus, Chikungunya virus, Italy, Italian Mosquito Control Association

#### MEDICAL IMPORTANCE OF CYCLORRHAPHAN TRUE FLIES (DIPTERA; INSECTA) AND THEIR RELATED STUDIES IN IRAN

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The dipteral allocate more than 10% of all biodiversity of the world. Cyclorrhapha or Muscomorpha is one of the three infraorder of suborder Brachycera. Their importance in human well being can be defined in two dimensions, their health treating effects and their beneficial aspects. Their health threatening affects are the results of the crossing of their feeding behavior with human.Mechanical transmissions of various pathogens and inducing of various kinds of myiasis are the most important threats on the human health. The study on mechanical transmissions of pathogens has been focused merely on Musca domestica. Majority of the studies on myiasis diseases are case reports and the comprehensive study on their dipteran agents are very low in Iran. Despite plenty of myiasis cases, unfortunately the disease is not recorded and reported in disease reporting system of Iran. The beneficial aspects of these true flies are their therapeutic effects and their informative effects. Employing their therapeutic effects such as Maggot Debridement Therapy has started in Iran less than one decade ago in laboratory. Mass rearing of Lucilia sericata, the main species using for MDT, is ongoing in Tehran University of Medical Sciences to be applicable for all required patients in Iran. The most precious information of these true flies is their use in estimation of Post Mortem Interval and some other measures in forensic investigations.

**Keywords:** Cyclorrhapha, myiasis, forensic entomology, maggot debridement therapy.





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MOLECULAR EPIDEMIOLOGY OF VISCERAL LEISHMANIASIS (VECTORS AND RESERVOIRS) IN IRAN

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The human Leishmaniasis is the most diverse and complex of all vector-borne diseases in their ecology and epidemiology. Leishmania infantum, belonging to the L. donovani complex, is the causative agent of human visceral (VL), cutaneous (CL) and canine visceral (CVL) Leishmaniasis in all Mediterranean countries. The disease is zoonotic and transmitted to humans from its reservoir hosts, mainly dogs by the bite of sandflies of genus Phlebotomus. Because of systemic parasite dissemination, VL is the most severe form of Leishmaniasis, which is nearly always fatal if left untreated. Phlebotomine sandflies (Diptera: Psychodidae) are the only vectors of Leishmania, and species of the genus Phlebotomus are the only known vectors in the Old World. Although >700 sandfly species have been described, only a few (~50) have been found to be able to support the development of Leishmania species and thus are vectors of disease. There are several reports on sporadic occurrences of zoonotic visceral Leishmaniasis in Iran, however, the disease is endemic in six provinces of Ardebil & East Azerbaijan (Northwestern), Fars, Bushehr and Kerman (South) and Khorassan Shomali (North eastern) of Iran. Three species of sand flies including: P. kandelakii, P. Perfiliewi transcaucasicus and P. tobbi have been reported to be vectors of the disease in north western and north eastern of Iran using molecular methods of PCR-RFLP. The species of P. (Paraphlebotomus) alexandri (Sinton) and P. (Lar.) major (Annandale) have been found naturally infected with L. infantum and are the VL vectors in the south part of the country. By employing of PCR, the species of P. (Paraphlebotomus)alexandri (Sinton) and P.(Lar.) major (Annandale) have been found naturally infected with L. infantum and are the ZVL vectors in the south part of the country. Phlebotomus (Lar.) keshishiani (Shchurenkova) and P. (Para.) caucasicus have been reported infected with promastigotes in southern Islamic Republic of Iran but no parasite identification was carried out.

Keywords: visceral leishmaniasis, vector, reservoir, *Phlebotomus*, *Leishmania* 

#### **ARTHROPOD-BORNE DISEASES IN IRAN**

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Arthropod-borne diseases are the main important health problem worldwide as well as Iran. Materials & Method: All the date was collected from authorities as well as published papers. There are some important arthropod-borne diseases in Iran including Malaria, Cutaneous Leishmaniasis, Visceral leishmaniasis, Crimean-Congo Hemorrhagic Fever (CCHF), and Tick relapsing fever. Furthermore scorpions are one of the risk factors for life in some parts, while other arthropodrelated diseases such as myiasis exist more or less across the country. Some probable Arthropod-borne disease in the future may be: Q-fever, Papatasi fever, Tularemia, Rift valley fever, Dengue fever, Yellow fever, West Nile viruses, Lactrodictism (spider bite), Plague, scabies, Nuisance insects of horseflies and Culicidae mosquitoes, Cockroach-borne diseases; damages by fire ants, blister beetles and bee stings. Also there is potential of diseases transmitted by black flies, rove beetles, lice and bedbugs.In order to combat these diseases, a comprehensive bilateral collaboration is requited through the country.

Keywords: arthropod-borne diseases, malaria, visceral leishmaniasis





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#### VECTORS AND RESERVOIRS OF CUTANEOUS LEISHMANIASIS WITH EMPHASIS OF RE-SEARCH PRIORITIES ON CONTROL OF SAND-FLIES IN IRAN

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Cutaneous Leishmaniasis is endemic in two forms, Anthroponotic Cutaneous Leishmaniasis (ACL) and Zoonotic Cutaneous Leishmaniasis (ZCL) in the country. About 20,000 cases of leishmaniasis (including ACL, ZCL and Zoonotic Visceral Leishmaniasis- ZVL) are reported annually but the real figures are 4-5 folds. Anthroponotic cutaneous leishmaniasis is still a neglected tropical disease in many parts of the country. Fourteen foci are active now in large and medium sized cities such as Tehran, Mashhad, Neishabur and Sabzevar in the northeast, Shiraz in the south, Kerman and Bam in the southeast, Yazd, Kashan and parts of the city of Esfahan in the central region. The parasite is *Leishmania tropica* and the vector is supposed to be Phlebotomus sergenti. The main reservoir host is human but dogs are considered as the secondary reservoir. Zoonotic cutaneous leishmaniasis is endemic in many rural areas of 17 out of 31 provinces and still is a great health problem in Iran. Four species of Gerbillinae rodents including Rhombomys opimus, Meriones libycus, Tatera indica and Meriones hurrianae act as main reservoir hosts in different parts of the country. Phlebotomus papatasi, is the only known vector. The control of the sandfly vectors of leishmaniasis is problematic because their larvae develop in large unknown terrestrial habitats making them impervious to available control measures. Furthermore, the behavior patterns of adults of different sandfly species are highly diverse, requiring tailormade control solutions based upon a profound knowledge of their biology. Possible lines of research in the battle against sand fly vectors are discussed in this short review.

Keywords: Leishmaniasis, ACL, ZCL, ZVL, sandfly, *Phlebotomus*, Iran

MOLECULAR VARIATION IN LEISHMANIA PAR-ASITES ISOLATED FROM SANDFLIES IN MO-SIAN, WEST OF IRAN

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Cutaneous Leishmaniasis (CL) is an important parasitic disease in Iran, considered as a public health problem especially in border of Iran and Iraq, Dehloran (Mosian region). The aim of this study was molecular identification of Leishmania parasites in sendfly as vectors of leishmaniasis. About 280 sandflies were trapped by sticky papers from 7 rural areas of Mosian in September to November 2012. All sandflies were identified using morphological characters of the head and abdominal terminalia. DNA was extracted from female sandflis and Leishmania was identified using PCR and sequencing. All of 280 trapped shadflies were identified as *Phelobotumus* Papatasi and Leishmania infections were detected in 1.7% out of 280 female sandflies. All Leishmania identified as L. major and submitted in GenBank as: LC014642.1, LC014641.1, LC014640.1 and LC014639.1. Abundance of of Phlebotomus papatasi and infection to L. major in studied regions showed that this vector is dominant in these areas.

Keywords: sandfly, L. major, Mosian





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Guilan, Iran; 20-22 May 2015

#### CLONING OF SAPOSIN-LIKE PROTEIN 2 (SAP2) GENE OF FASCIOLA HEPATICA IN EXPRESSION VECTOR

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Fasciola hepatica is the causative agent of liver fluke disease (fasciolosis) in cattle and humans. This infection has recently been recognized as an emerging or re-emerging zoonotic disease, with worldwide economic losses of approximately \$2 billion annually. Fasciola hepatica saposin-like protein 2 (FhSAP2) is a novel antigen isolated from the adult worm, and belongs to the saposin-like protein (SAPLIP) family. It is demonstrated that FhSAP2 is a good antigen for antibody detection in acute and chronic phases of human fascioliasis and highly reactive with sera from experimental animals within 2 weeks post infection. The main objective of present work was cloning of SAP2 Gene of F. hepatica in an expression vector. DNA extraction of Fasciola hepatica was based on Chelex® method. The SAP2 fragment amplified with specific primers. The purified PCR products were ligated between the Nde1 and Xho1 sites of the pTZ57R/T cloning vector and transformed into Escherichia coli DH5a strain. Then the PCR product was digested with Nde1 and XhoI and it was subcloned into pET-28b (+) expression vector which digested with the same enzymes. The plasmid was purified and approved by electrophoresis and enzyme restriction. After isolation from pTZ57R/T, it was subcloned into pET-28b (+) plasmid. After enzyme restriction and electrophoresis, a fragment of 270bp separated from pET28b.Recombinant plasmid of SAP2 gene was constructed for further study, suggesting its potential use in the serologic diagnosis of F. hepatica infections in humans. It seems that, this antigen is a suitable candidate for diagnostic kits. Production of recombinant parasite's antigen has many advantages such as being cost effective and standardizes serological tests.

Keywords: Fasciola hepatica, FhSAP2, cloning, expression vector

### THE EFFECT OF METRONIDAZOLE IN TREAT-ING HUMAN FASCIOLIASIS

#### Fariborz Mansour-Ghanaei\*, Afshin Shafaghi, Mohammadsadegh Fallah

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Triclabendazole is drug of choice for the treatment of human fascioliasis but many cases do not respond to this drug. The aim of the present study was to determine the effect of metronidazole in patients who did not cure after treatment with triclabendazole, in Guilan. This open self-controlled trial was performed from 1999 to 2001 in the outpatient clinic of Gastrointestinal & Liver Diseases Research Center in Guilan. Patients, who passed Fasciola eggs in stool and had positive serum anti Fasciola antibody (ELISA), at least three months after treatment with triclabendazole, were enrolled and received 1.5 g/day metronidazole orally for three weeks. Two months and 12 months after end of therapy, stool examination in 3 consecutive days and serum anti Fasciola antibody test were performed. Frequency of patients with negative serology for Fasciola and/or absence of Fasciola egg in stool were determined. Forty-six patients, 26 females and 20 males, were enrolled with mean ( $\pm$ SD) age of 34.6 ( $\pm$ 9.8) years. Two months after end of therapy, stool exam became negative in 35 patients and in 31 patients became negative both in serology and stool examination. Difference in response to treatment between age groups and genders was not significant. All patients with abdominal pain became pain free following therapy. Most frequent side effects were metallic taste in 14 (30.4%), headache in 8 (17.4%) and nausea in 6 (13%). 12 months after end of therapy, 28 out of 35 patients were examined again and all were negative both for serology and egg in stool examination. Metronidazole, 1.5 g/day for 3 weeks, seems to be an effective, available, well-tolerated alternative for treatment of human fascioliasis.

Keywords: human fascioliasis, metronidazole, triclabendazole





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Guilan, Iran; 20-22 May 2015 HUMAN LIVER FLUKES: FROM PATHOGENESIS We pioneer

# TO CONTROL

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Human liver fluke diseases caused by Opisthorchis viverrini, O. felineus and Clonorchis sinensis are major foodborne trematodiases in Southeast Asia, the Far East, western Siberia and eastern Europe. The infections are associated with several hepatobiliary diseases, including inflammation (cholangitis), cholecystitis, gallstones, hepatomegaly, periductal fibrosis and cholangiocarcinoma (CCA), the highly fatal bile duct cancer. Throughout Southeast Asia, particularly, there is a strikingly high incidence of CCA in regions where the liver fluke, O. viverrini, infection is endemic. In Thailand, rates of CCA in regions where the parasite is endemic are unprecedented CCA is responsible for about 15%-25% of liver cancers in the USA but represents 86.5% of the cancers in Khon Kaen Province, the highest incidence in the world. O. viverrini is classified as Group 1 carcinogens by the International Agency for Research on Cancer, World Health Organization. Liver fluke infection induces severe inflammation of the bile ducts, resulting in oxidative and nitrative DNA damage of the biliary epithelium. Surprisingly, however, affected cells may not undergo apoptosis. Experimental infections of hamsters with O. viverrini have corroborated the findings in human infections. Opisthorchis antigens are observed in infected biliary epithelium and associated with intense inflammatory cell infiltration. More indepth mechanism, we found that releasing of proinflammatory cytokine/chemokine, specifically IL-6 and IL-8, through biliary TLR4 activation in a MyD88-independent and dependent manner, respectively supporting inflammatory mechanism of the infected bile ducts. Elevation of Opisthorchis specific IL-6 is also observed in peripheral blood mononuclear cells from infected individuals with advanced periductal fibrosis. IL-6, a multifunctional proinflammatory cytokine, can induce inflammation, anti-apoptosis, cell transformation and eventually malignancy. Excretory-secretory (ES) products of the liver flukes can stimulate cell proliferation in vivo and in vitro demonstrating that the parasite ES products are mitogenic. We recently found that O. viverrini ES products completely inhibited biliary cell apoptosis when cells underwent oxidative stress. This suggests that O. viverrini -ES may play role in neutralizing oxidation in infected biliary epithelium rendering them escaping from apoptosis, in addition to effect of IL-6. Inflamed biliary epithelium with oxidative DNA damage cannot undergo apoptosis but stimulated to hyper-proliferate, providing an additional potential mechanisms by which biliary epithelial cells become neoplastic in liver fluke-associated CCA. Apart from the pathogenesis of the liver fluke infection, we also have worked on liver fluke control, having developed an extensive community-based program during the past few years.

We pioneered the introduction of integrated opisthorchiasis control using an EcoHealth/One Health approach at Lawa Lake, a field research site where the disease is endemic. A program has been carried out for over 5 years using chemotherapy, novel intensive health education methods both in the communities and in schools, ecosystem monitoring and active community participation. As a result, the infection rate in more than 10 villages surrounding the Lake has declined to approximate one half of the average of 60% as estimated by a baseline survey four years ago. Strikingly, the Cyprinoid fish species, which are the intermediate host, now showed less than 1% prevalence compared to a maximum of 70% during the baseline survey. This liver fluke control program, named "Lawa model," is now recognized nationally and internationally. The Lawa village and its community hospital has become the training site for integrated liver fluke control for health personnel from all over Northeast Thailand. The "Lawa model" is now being expanding to other parts of Northeast Thailand and neighboring Mekong countries.

Keywords: human liver flukes, Lawa model, opisthorchiasis, Thailand





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#### DIFFERENTIATION OF FASCIOLA HEPATICA AND FASCIOLA GIGANTICA BASED ON THEIR ISOENZYMATIC PATTERNS

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Morphological methods alone are not sufficient for differential diagnosis of Fasciola hepatica and Fasciola gigantica. These two species differ in their method of transmission and epidemiological characteristics and each species is transmitted by a certain species of Lymnaea snails. For the prevention and control of fascioliasis, it is important to differentiate these two species. Isoenzymes are different molecular forms of the same enzyme that have different migration speeds in electrophoresis. Isoenzyme analysis is a precise and effective diagnostic method which accurately distinguishes the species of organisms. This study aimed to compare the isoenzymatic patterns of Fasciola hepatica and Fasciola gigantica for differentiation of these two species. Fasciola hepatica and Fasciola gigantica samples were collected from infected liver of sheep and cattle from the local slaughterhouse. Fasciola species was determined by molecular method (PCR-RFLP), using ITS1 primers. The worms were crushed in PBS by tissue grinder and were washed twice with PBS and centrifuged at 3000 RPM. Stabilizers (dithiothreitol, aminocaproic-acid, EDTA) were added to the pellet and after several cycles of freezing and thawing, the precipitate was centrifuged and the supernatant was collected. Enzyme electrophoresis was done, using discontinuous polyacrylamide gel electrophoresis and the activities of five enzyme systems including Isocitrate Dehydrogenase (ICD), Glucose 6-Phosphate Dehydrogenase (G6PD), Superoxide Dismutase (SOD), Malate dehydrogenase (MDH), and Glucose Phosphate Isomerase (GPI) were evaluated and compared in both Fasciola species. Result: The zymodemes obtained from SOD system in F. hepatica were two bands with relative motilities of 0.25 and 0.55, respectively while in F. gigantica were four bands with relative mobilities of 0.27, 0.42, 0.55, and 0.65, respectively. In ICD enzyme system, one band with relative mobility of 0.26 and one band with relative mobility of 0.21 were observed in F. hepatica and F.gigantica, respectively. G6PD system revealed one band with relative mobility of 0.29 in F. hepatica and one band with relative mobility of 0.25.in F. gigantica. GPI revealed one band in F. hepatica and F. gigantica with relative mobility of 0.39 and 0.30 respectively. Isoenzymatic pattern of MHD was the same in both species where they produce 3 bands with relative mobilities of 0.05, 0.12 and 0.21. Findings of this study showed that F. hepatica and F. gigantica have entirely different isoenzyme patterns in the enzymes of Isocitrate Dehydrogenase (ICD), Glucose 6-Phosphate Dehydrogenase (G6PD), and Superoxide Dismutase (SOD). These enzyme systems may well be used to distinguish the two species of Fasciola from each other.

Keywords: Fasciola hepatica, Fasciola gigantica, Isoenzyme pattern, Lymnaea QUANTIFICATION OF THE DIFFERENT DE-VELOPMENTAL COURSES OF FASCIOLA HEPAT-ICA AND F. GIGANTICA IN VARIOUS HOST SPE-CIES IN A ZONAL OVERLAP ENDEMIC AREA OF NORTHERN IRAN

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Fascioliasis is an emerging or re-emerging parasitic disease caused by fasciolid trematodes, mainly affecting the biliary canals and gallbladder of herbivorous ruminants worldwide. In the last two decades, this disease has proved to also be of highly public health importance, with the description of human endemic areas in Europe, Americas, Asia and Africa, and an increasing number of human reports in many countries of all continents. Depending on the endemic area and causal agent- Fasciola hepatica and F. gigantica- fascioliasis shows different epidemiological, pathological and control characteristics. Classically it has been accepted that F. hepatica is present worldwide, while the distribution of two species overlaps in many areas of Africa and Asia, including the endemic areas of Guilan Province, northern Iran. This is the first study in the world in which a detailed distribution of both Fasciola species is analysed in a human fascioliasis endemic area with a zonal overlap transmission pattern. An accurate analysis was conducted to phenotypically discriminate fasciolids from naturally infected livestock (cattle, buffaloes, sheep and goats). The distribution of the % F. hepaticalike and F. gigantica-like flukes detected in each liver versus altitude (m) in each group was analysed. The presence of the fasciolids in different locations, below sea level to altitude above 1000 m, is noteworthy. A significant positive correlation was obtained between altitude and % of F. hepatica and a significant negative correlation was obtained between altitude and % of F. gigantica. The results show that F. gigantica population in cattle, buffaloes and sheep share large size values, but smaller specimens are present mainly in lowland populations located below sea level, independently of host species (cattle, buffalo).Based on the results, the influence of the host species on the size and shape of F. hepatica-like and F. gigantica-like specimens does not follow the same pattern. It means that, F. gigantica-like global size are influenced by the host species when comparing liver flukes from sheep and bovines, while in the case of F. hepatica-like specimens this influence is less evident. Concerning global shape, the influence of host species is similar in both fasciolids when comparing liver flukes from sheep and bovines.

Keywords: Fasciola hepatica, Fasciola gigantica, intermediate forms, altitudinal relationships, geographical distribution, host influence, Guilan, Iran





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#### THE EPIDEMIOLOGY OF HUMAN FASCIOLIASIS IN GUILAN PROVINCE AND EVALUATING THE RECENT REPORTS ON NEW FOCI OF HUMAN DISEASE IN IRAN: AN UPDATE

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The global public health importance of human fascioliasis has increased during last few decades due to the appearance of new emerging and re-emerging foci in many countries worldwide. World Health Organization has included Iran among those countries having serious problem with fascioliasis. The highest reported cases of human disease in Iran belong to Guilan province, where two world largest ever outbreaks have occurred affecting more than 15000 individuals. Recently, new foci of human disease have been reported from various part of the country. The status of human fascioliasis in Iran is discussed here. The situation of the disease in Guilan province was analyzed using epidemiological studies on human populations, host reservoirs and snail intermediate hosts. The identity of fasciolids and their intermediate hosts were characterized by molecular methods. The new foci of the disease in different provinces have mainly been reported based on serological tools. Both F. hepatica and F. gigantica and intermediate forms are prevalent in Guilan province. The distribution of F. hepatica and F. gigantica, and the snail intermediate hosts are quite different based on various geographical situations. F. hepatica and Galba truncatula are more prevalent in highlands while F. gigantica and Lymnaea (Radix) auricularia are prevalent in lowlands. Based on the results of a community-based study human fascioliasis is hypoendemic in human population of Guilan province. In the past few years several new foci of human disease from provinces of Kermanshah, Kohgyluye va Boyerahmad and Ardabil and various sporadic cases from different provinces of the country have also been reported. Although human fascioliasis is hypoendemic in Guilan province, due to the appropriate conditions of disease transmission and potential for occurrence of large outbreaks, Guilan province should be considered as an endemic zone having serious problem with fascioliasis and the passive control measures, based on WHO, should be performed for disease control. To verify the reports on new foci of human disease in other provinces of the country comprehensive standard studies is recommended.

Keywords: human fascioliasis, endemicity, epidemiology, Lymnaea, Iran.

#### IMPACT OF CLIMATE AND GLOBAL CHANGES ON FASCIOLIASIS IN SOUTHERN ASIA: THE PUNJUB AS A MODEL FOR MATHEMATICAL AND REMOTE SENSING-GIS ASSESSMENT

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Southern countries of Asia top climate change's most vulnerable list. In southern Asia, human fascioliasis is giving cause for concern due to the spread of the fasciolid fluke species throughout and the emergence of human endemic areas in its two geographically extreme areas of the Near East and South East. According to the Global Climate Risk Index (CRI) 2012, Pakistan ranked 8th in the world country list according to the Long-Term Climate Risk Index for the 1991-2010 period and first in the world country list concerning 2010. Pakistan is one of the 16 countries rated within the highest risk category by the Climate Change Vulnerability Index (CCVI). CCVI enables to identify areas of risk by evaluating 42 social, economic and environmental factors to assess national vulnerabilities across core areas. According to the CCVI 2011 map, Pakistan enters in the extreme risk category mainly due to the Punjab province, which shows the highest CCVI risk almost throughout its whole area. Abiotic and biotic environmental characteristics linked to the requirements of fascioliasis transmission in the Punjab Plain, Pakistan, are analyzed as a model to assess human and animal fascioliasis risk in lowlands of southern Asia and its trends related to the impacts of both climate change and global change. Two climatic forecast indices and a remote sensing marker are used to characterize the climatic factors and the earth surface in order to ascertain the epidemiological complexity and time-lag dynamics of fascioliasis. The present study shows that forecast climatic indices and remote sensing tools may become the second line markers for this disease. In fact, the combination of Mt, Wb-bs, NDVI and index/prevalence correlation time-lags here used has proved to be a useful approach to assess fascioliasis in an endemic area of the highest epidemiology and transmission complexity. Results show how man-made land modifications may impact on fascioliasis, up to the level of (i) allowing the disease to be transmitted in areas where a priori neither climatic conditions nor the land features are those required, (ii) pronouncedly changing the epidemiological characteristics of the disease in both humans and livestock, and (iii) opening the possibility for the disease to expand in south Asian lowlands. Moreover, climate change is shown to be able to modify the characteristics of fascioliasis transmission and epidemiology affecting both humans and livestock in the Punjab Plain and whole Pakistan. These results suggest potential situations of concern in other south Asian countries in the near future.

Keywords: fascioliasis, global changes, climate changes, sensing-GIS, Punjub





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IDENTIFICATION OF GIARDIA, ACANTHAMOE-BA AND ENTAMOEBA SPP FROM RIVER WATER SAMPLES OF TEHRAN AND GUILAN PROVINC-ES, IRAN

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Introduction & Objectives The parasites, Giardia, Acanthamoeba and Entamoeba spp are isolated from samples of patients from different parts of Iran, but due to difficulties in identifying these parasites in water samples, there are very few studies conducted on water samples. So this study designed to identify these parasites in some surface waters in Iran. In this study, 36 surface water samples collected from Guilan and Tehran provinces. Samples concentrated by IMS and/or by SF methods. Finally they were examined for Giardia, by semi nested PCR and IFA. Acanthamoeba and Entamoeba spp detected using PCR method. PCR were carried out in positive samples for genotyping of Giardia spp and Acanthamoeba isolates. The results showed that 40% and 47% of the samples were positive for Giardia spp by IFA and nested PCR respectively. Acanthamoeba and Entamoeba spp (E. histolytica, E. dispar and E. moshkofski) were detected in 85% and 20% of samples respectively. All of Giardia isolates belonged to genotype B and Acanthamoeba belonged to T4 and T5. This is the first study in which three pathogenic parasites, Giardia, Acanthamoeba and Entamoeba spp, have been isolated from the same water supplies. These results indicate that river water supplies in study areas were contaminated by pathogenic species of Giardia spp. Acanthamoeba and probably Entamoeba spp, so the potential risk of waterborne protozoan epidemics in humans and animals should be considered.

Keywords: Giardia lamblia, Acanthamoeba, Entamoeba, PCR, water, genotyping, Guilan, Tehran

PHYLOGENETIC ANALYSIS OF GIARDIA DUO-DENALIS SUB-ASSEMBLAGES IN PATIENTS IN FARS, IRAN

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Molecular genetic studies have demonstrated that G. duodenalis is a species complex with seven major genotypes or assemblages (A-G). Two distinct genetic assemblages A and B infect human and a wide range of domestic and wild mammals. The objective of this study was the genotyping and subtyping of G. duodenalis isolates in Fars province, south of Iran. Human fecal samples associated with the relevant clinical symptoms of Giardiasis were collected from patients at the health centers and hospitals in Shiraz, Fars province. Microscopic conformation of G. duodenalis, Purification and DNA extraction were done. Semi-nested PCR was performed using the primers (GDHeF, GDHiF and GDHiR). Phylogenetic analysis of 40 gdh nucleotide sequences of G. duodenalis isolates obtained in this study and the known sequences isolates published in GenBank was determined to further clarify the relationship of the different genotypes to each other. Phylogenetic analysis was carried out using Neighborjoining (NJ) in the program MEGA4. Reference gdh gene sequences from each of G. duodenalis genotypes (AI, AII, BIII, BIV, C, D, E, F, G) from GenBank accession numbers were L40509, L40510, AF069059, L40508, U60984, U60986, U47632, AF069057, AF069058 respectively). Phylogenetic analysis has shown Fars isolates were widely distributed within assemblage A cluster (sub-assemblage AII). The remaining Fars isolates were dispersed throughout the assemblage B cluster (sub-assemblages BIII and BIV). No samples were joined in clusters corresponding to sub-assemblage AI, assemblages C, D, E and F and G of G. duodenalis. The phylogenetic tree indicated that assemblage A was the most frequent, corresponding to 32 cases that were distributed in sub-assemblage AII. The second cluster was contained the human assemblage B isolates with 8 cases of the studied samples. Two major lineages (sub-assemblages BIII and BIV) were found in assemblage B. Third and fourth clusters were the dog assemblages C or D. The fifth cluster contained the cattle, sheep assemblage E. The sixth cluster contained the cat assemblage F cluster. The seventh contained the rodent assemblage G cluster. Finally the eighth cluster contained the G. ardeae. Phylogenetic analysis of the gdh gene of G. duodenalis isolates from patients provided strong bootstrap support for the placement of genotypes A and B in separate clusters, indicating the differentiation of the assemblages A and B. The high prevalence of sub-assemblage AII based on Phylogenetic analysis suggested anthroponotic transmission from human to human of the main causes of Giardiasis in this area.

Keywords: Giardia duodenalis, phylogenetic, assemblages, Iran





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#### IDENTIFICATION OF GIARDIA SPP. PROTOZO-AN IN HOUSING REFINERY FILTERS IN ABA-DAN CITY, SOUTHWEST IRAN

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Water is the most important element for life. For this case, preventing of water pollution is equally important. According to CDC, various microorganisms including Giardia spp. can be transmitted by different sources of water and infect human beings. The aim of this research was to study the existence of Giardia in drinking water in Khoramshahr, Iran, before, after and in filters of household refinery system by direct examination of precipitation and Touchdown- PCR. Twenty samples were collected randomly from four districts of the city (5 samples of each district, North, South, East and West). The samples were collected by referring to the houses using refinery filters. Three samples were collected from each filter, one liter of pre- filtration and one liter post-filtration and the old filter was removed, then new filter was installed. The samples of the water and filters were used for examination. Totally 60 samples were collected from 20 houses. The samples were centrifuged and the precipitation was examined by direct smear preparation and Touchdown- PCR. In direct examination, 4 (20%) out of 20 precipitate and by Touchdown- PCR method 6 (30%) out of 20 from the filters were positive for Giardia spp. protozoa. The results of this study indicated that Giardia spp. is present in 60% of drinking water of Khoramshahr city, and it is suggested that all household should use water refinery system and obtain safe drinking water.

Keywords: Giardia sp, drinking water, refinery system, Abadan, Iran

#### ALVEOLAR HYDATID CYST IN PIKAS (OCH-OTONA RUFESCENS) IN CHENARAN AREA, KHORASAN RAZAVI PROVINCE

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Alveolar hydatid cyst is a dangerous zoonotic disease. Echinococcus multilocularis is the main agent of disease that lives in the small intestine of canidae family. Wide ranges of animal, such as rodents are intermediate hosts. The E. multilocolaris has been reported in foxes of Iran. Recently, high percentage (75%) of alveolar hydatid cyst was reported in Pikas (Ochotona rufescens) in Chenaran area using molecular method. Due to the importance zoonotic alveolar hydatid cyst, the aim of study was conducted to comfirm the presence of alveolar hydatid cyst in pikas in Chenaran area. From April to September of 2014, five parts of Chenaran area with mountainous region were selected, and then 10 pikas were trapped in each part with collaboration of environment department. The carcasses were transferred to parasitological department in cold condition. After necropsy, the viscera was carefully observed and sampled from the infected tissues with cyst or similar cyst forms, for parasitological, pathological and molecular examination. The molecular examination was only done on the positive samples. Fifty euthanized pikas, cyst forms were found in liver and lungs of three pikas. The parasitological examination only showed the strobilocercous infection in one of infected liver of pika. No alveolar cysts and lesions related to infection were observed in the microscopic sections in the suspected cases.Based on the parasitology and pathology finding, alveolar hydatid cysts were not observed in any pikas. The results also showed that the molecular examination is not enough to diagnosis alveolar hydatid cysts and final diagnosis requires parasitological and pathological examination in intermediate hosts as well.

Keywords: survey, alveolar hydatid cyst, Pika, Iran





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A RETROSPECTIVE STUDY ON 182 CASES OF HUMAN HYDATIDOSIS BASED ON HOSPITAL RECORDS, FROM 2006 TO 2013 IN HAMADAN, WEST OF IRAN

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Hydatidosis is a zoonotic disease common in the Middle East countries and Eastern Mediterranean region. Iran is classified as an endemic area for hydrid disease according to WHO classification. In humans, hydatidosis leads to economic losses due to treatment costs and lost wages and even death from surgery side effects in some cases. The reservoir and intermediate hosts of Echinococcus granulsus and human hydatidosis are more prevalent in the Alborz and Zagros Mountains slop as two rich and good pastures for livestock. A 7-year (2006-2013) retrospective study was carried out to investigate the occurrence of hydatidosis in patients at main hospitals in Hamadan Province, Iran. This study reviewed the files of patients with hydatid cysts treated surgically in the main public and private hospitals in Hamadan province during 2006 and 2013. The data extracted from 8 hospitals in the public and private sectors. Demographic data, diagnostics measures including laboratory and radiological findings, clinical manifestations, site of the cyst, surgical approaches, and outcome were noted in the prepared checklist and analyzed. Hospitalization records were reviewed and confirmed cases of hydatid cyst were classified according to ward of residence, age, sex, cyst location and type. A total of 182 hydatidosis patients were diagnosed and operated giving an average of 26 cases per year, equivalent to approximately 15 cases per 1000,000 people per year. High proportion of cysts (70.9%) occurred in the liver, 24.7% in the lung and 2.2% in both organs; and females had higher cysts (52%) than males (48%). Mean age of patients were 44.5 years at range of 3 to 91 years. Most of the cysts were at the fourth decade of the life. Majority of the patients were illiterate (32.2%) and residence in the rural areas (61.7%) and were housewives (36.8%). Most of the patients were from Hamadan followed by Malayer. Finally, almost 90% of patients diagnosed by CT and 8% had history of surgery for cyst. These findings indicate hydatidosis as a major health problem in this area and more extensive epidemiological investigations of CE is necessary to better determine the prevalence, economic impact and risk factors for the disease in this province and other provinces of Iran as well.

Keywords: hydatidosis, surgery, hospital, Iran

#### IN SILICO ANALYSIS FOR IDENTIFYING PO-TENTIAL MICROSATELLITES REGIONS IN GE-NOME OF ECHINOCOCCUS SPP.

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Microsatellites are repetitive stretches of nucleotides that are non-randomly distributed as single base pair stretches or as a combination of two to six nucleotides units within coding and in non-coding regions of the genome. Microsatellites are very useful for developing genetic studies widely used in linking genome sequence with traits, diversity investigation, mapbase cloning and molecular breeding. In silico, development of microsatellites of genomes has brought them up as potential markers for transferability among the species, ease of development and as key players in genome length variation. The increasing whole sequences of more and more large genomes provide sources for microsatellites mining in silico. The genus *Echinococcus* includes six parasite species of cyclophyllidea tapeworms to date, of the family Taeniidae. Infection with Echinococcus results in hydatid disease, also known as echinococcosis. Echinococcus spp., genomes was downloaded from GeneDB database along with its related RefSeq dataset from NCBI. Then, for prediction of potential microsatellites regions, we used two softwares, GMATo and Chloro-MitoSSRDB. In this study, in silico analysis was used for prediction microsatellites regions in Echinococcus spp., genomes. We found 112 highly potential imperfect and 4 perfect microsatellites sequences. This is the first genome survey report of Echinococcus spp., based on a computational approach for microsatellites regions finding. For more validation of these regions, we recommend using the experimental methods.

Keywords: computational prediction, microsatellite, tapeworms, *Echinococcus* spp.





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#### MORPHOLOGICAL AND MOLECULAR CHARAC-TERIZATION OF ECHINOCOCCUS GRANU-LOSUS ISOLATED FROM CATTLE HYDATID CYSTS IN KHORRAMABAD, LORESTAN PROV-INCE, IRAN

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Hydatidosis is one of the zoonotic diseases, which affect animals and human beings at the larval stage of Echinococcus granulosus, thereby playing a role in exacerbating the economic and health problems in Iran. This study was conducted to investigate the strains of Echinococcus granulosus isolated from cattle hydatid cysts, in Khorramabad, Lorestan province, west of Iran. Thirteen isolates of hydatid cyst of cattle from lung (12 samples) and liver (1 sample) organs were collected from Golshan slaughterhouse, in Khorramabad. All of the samples were transferred to the laboratory for morphometric characterization and molecular study. In morphological characterization, blade length of large (LBL) and small (SBL) hook and the ratio of blade length to total length in large (LBL/LTL) and small (SBL/STL) hooks and total length of large (LTL) and small (STL) hooks were measured. In molecular study, a partial sequence of cytochrome oxidase 1 (COX1) with 440 bp in length was amplified applying primers J3 and J4.5. Genomic DNA sequencing was performed by Sanger's method. The morphological results showed that there is no significant difference between isolated from cattle hydatid cyst and G1 strain (P>0.01). The results of molecular studies support the findings of morphological characterization. All sequences showed 100% identity with G1 strain. The results of this study showed that G1 strain is a causative agent of cattle hydatid cyst in Khorramabad. On the other hand, the cattle play a role in enabling the parasite to complete its cycle. Hence, in order to execute a control program for minimizing the effects of this disease, the cattle should be considered as a source of infection for dogs. The results of this study could be helpful in designing such a control program in the region.

Keywords: *Echinococcus granulosus*, cytochrome oxidase I, hydatidosis, Khorramabad

#### THE EPIDEMIOLOGIC STUDIES ON CYSTIC ECHINOCOCCOSIS IN TURKEY

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The data about the prevalence of cystic echinococcosis (CE) may not be considered reliable enough in many countries such as Turkey, as most of them were collected from the limited hospital records. Radiological imaging, serological tests, and pathological examination of biopsy materials are generally used in individual diagnosis, while ultrasound scanner (US), serology, and chest radiology may be used in community-based screening surveys of CE. A four-staged study in primary school children was planned to work, especially on the epidemiology of CE in Manisa, a province located in the western part of Turkey. The first stage was carried out to evaluate the efficacies of portable US, serology and chest X-ray. Of the 630 children examined for CE by these methods, hepatic CE was detected in two cases (0.3%) by US. US was found to be more reliable and simple compared to others in field studies. Portable US were used alone in the second stage in another pilot region. Three of 575 children (0.5%) were diagnosed with CE (two hepatic and one renal involvement) by US alone. In the third stage; an epidemiological province-based field study with portable US was performed using sampling method in a province, for the first time. A total of 6093 children from 37 primary schools were selected as the representative sample of the total number of 166,766 children and examined by the US. Nine children were diagnosed with CE and CE prevalence was found to be 0.15% in primary school children. In the fourth stage; it is planned to repeat the study at the same schools in eight years' time, in order to evaluate the efficacy of the control programs, since nearly all students involved in this study will be graduated within this period. A similar study was performed in Elazığ, a province in East Turkey in which sheep rising is more common than Manisa. A representative sample of 2500 primary school children were scanned by portable US while standing, and the prevalence was found to be 0.2% (6/2500). In our another study, a total of 4275 students from Celal Bayar University, Manisa, Turkey, were screened by ultrasonography (US) and specific antibodies for CE were examined by Western blotting (WB) and ELISA in finger prick blood samples of 2034 of 4275 volunteered students. Six new cases were diagnosed as CE by US, and in addition to these cases, three students were also detected to have been previously operated and pathologically confirmed for hepatic CE. The prevalence of CE by US was calculated as 0.21% (9/4275) among university students in Manisa. Due to the increase in the prevalence with age, the infection is thought to be an important public health problem in Turkey. As a reliable, simple, inexpensive and rapid method, portable US was found to be more useful in the diagnosis of hepatic CE in field studies, than serological tests, which may cause false positivity and discrepancy in results. Chest X-ray is accepted as the best way for pulmonary cysts. Our results also supported that WB is rather difficult and not feasible as a mass screening test and may not be effective for confirmation especially in asymptomatic cases. As a result, we recommend US to be used initially in mass screening surveys for CE followed by confirmation by ELISA for suspected cases. Further examination primarily by chest X-ray followed by computed tomography and/or magnetic resonance imaging, if needed, should be recommended for US negative, ELISA and WB positive individuals who may have non-abdominal cysts.

Keywords: cystic echinococcosis, epidemiology, Turkey





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ALVEOLAR ECHINOCOCCOSIS: NEW ASPECTS OF EPIDEMIOLOGY AND CONTROL

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Alveolar echinococcosis (AE) is one of the most pathogenic parasitic zoonoses in the Northern Hemisphere and continues to have very serious clinical implications and a high burden of disease despite improvements in diagnosis and treatment. The endemic area of E. multilocularis is still expanding but not well documented in many regions. In Europe, growing red fox populations and the colonisation of residential areas by foxes are major factors for the urbanisation of the parasite life cycle and the corresponding significantly increased infection risk for a larger human population. Several studies documented an increase of human AE incidences in Central Europe, the Baltic region in Northern Europe, in Kirgizstan and especially in China. In Switzerland, an average twofold increase of the annual incidence was documented in 2000, around 10-15 years after fox populations had significantly increased due to the successful control of rabies. Canid species (especially the red fox and the raccoon dog, Nyctereutes procyonoides) represent the major definitive hosts of E. multilocularis, with prevalences up to 70%. Dogs in Europe usually have lower prevalences (< 1%), but they nevertheless can substantially contribute to the E. multilocularis egg contamination of the environment because of their high population densities especially in urbanised areas. An even more prominent role of the domestic dog in the zoonotic transmission has been described for endemic areas in China and Kirgizstan. Intervention strategies to minimise the infection risk of AE for humans have to consider the complex parasite-wild host ecology, the possible involvement of dogs, and they have to foresee the effects of planned human-wildlife interactions such as increased hunting pressure. One promising approach for parasite control is the deworming of definitive hosts with praziquantel-containing baits. This strategy was used in defined rural and urban areas, and a significant reduction of the egg contamination was recorded within 1-2 years. The continuous control of E. multilocularis on a larger scale by baiting foxes is expensive. Thus, due to the long incubation period of AE in humans, a cost benefit of such a strategy can be expected only after around 20 years.

Keywords: alveolar echinococcosis, epidemiology, control

FIRST MOLECULAR IDENTIFICATION OF SAR-COCYSTIS CRUZI IN A HUMAN AS DEFINITIVE HOST

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Several opportunistic protozoan parasites have been found as causative agents of serious intestinal infections in patients with the immunodeficiency diseases. Sarcocystis is not usually considered as an important enteric pathogen in immunodeficient persons, but it might be expected that species for which humans are the final host would be encountered increasingly often in immunodeficient persons. To determine the incidence rate of intestinal parasitosis in 741 immunodeficient patients with recurrent persistent or chronic diarrhea, fecal examination were performed by using formalin-ethyl acetate sedimentation and modified acid-fast staining procedures in the enteric protozoology laboratory of Shiraz University of Medical Sciences, Shiraz, Iran. During microscopic examinations of specimens, regardless of presence of other intestinal parasites, sporocysts of Sarcocystis were seen in fecal sample collected from a woman with AIDS and CD4 count less than 100 cells/µL. A semi-nested polymerase chain reaction (PCR)-sequencing analysis of the small subunit-ribosomal DNA (SSU-rDNA) gene was used to recognize Sarcocystis species. Used molecular method identified the species as Sarcocystis cruzi.In the present study, the first molecular identification of S. cruzi from an immunocompromised human as definitive host is documented.

Keywords: Sarcocystis cruzi, diarrhea, AIDS, PCR





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#### WATER SOURCES CONTAMINATION AND WA-TER BORNE PROTOZOAN DISEASES IN IRAN

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Recently, there is an increase in epidemics of water borne protozoan diseases. These parasites are isolated from samples of patients from different parts of Iran, but due to difficulties in identifying these parasites in water samples, very few studies have been carried out in water samples. This study designed to investigate these parasites by different Filtration, Purification and Detection methods. 50 surface water samples collected from Iran, 10 L of each river water samples were filtered through membrane filters (diameter 142 mm) or capsule filter. After purification by IMS or flotation methods, substances were checked for Cryptosporidium, Giardia, Entamoeba spp. and Toxoplasma by PCR and/or Real time PCR, LAMP and IFA assays. Cryptosporidum, Giardia, Entamoba spp. and Toxoplasma oocyst were detected in many samples of surface waters. Sequencing result approved presence of pathogenic species of Cryptosporidium and Giardia species in the study area. IFA revealed high levels of Giardia and Cryptosporidium oocysts in water samples. The study reveals that the investigated water supplies were contaminated by these pathogenic parasites. Therefore, there is a potential for outbreak of diseases. Therefore, we must design and establish equipment for monitoring these protozoans in water source to prevent epidemics of these diseases. However, each method has its own advantages and disadvantages. So based on the aim and the study design, a combination of detection methods should be applied.

Keywords: Cryptosporidium, Giardia, water, IMS, PCR

PARASITES IN DOMESTIC SEWAGE WASTE AND MANURES AND THEIR CONTROL

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Soil transmitted protozoa and helminths of people and animals remain a continued source of infection via the contamination of food items. Besides the human helminths (Ascaris lumbricoides and Taenia spp.) and the protists Cryptosporidium hominis and Cryptosporidium parvum, there are other organisms from sewage and manure that can pose threats to the health of humans and animals, including: Cyclospora, Toxoplasma, Balantidium and possibly Blastocystis and Giardia. Numerous trials have been carried out on various methods to reduce monitor and reduce parasites in human sewage sludges and manures (cattle and swine) in many different types of facilities. These have utilized physical, chemical, irradiation, and sonication modalities. The problems with all methods remain the cost-benefit ratio of implementation and the work required maintains the systems. The work in our laboratory has concentrated on two agents specifically, Ascaris suum (a surrogate for Ascaris lumbricoides and considered by the US Environmental Protection Agency to be the most difficult to inactivate helminth egg) and Cryptosporidium parvum of calves because of its omnipresence and zoonotic potential. Heat is a very successful means of inactivation, and when combined with drying is certain to inactive the eggs if the temperature is greater than 60C for just a few minutes. This can easily be obtained in well-controlled compost systems, and with care, it can be produced in aerated static piles. Long-term liquid storage has the potential to inactivate the oocysts of Cryptosporidium spp. but has very little effect on the eggs of Ascaris. Various work with short-chain fatty acids have also shown that they can have high levels of inactivation of eggs and the resistant stages of protists if the chemistry of the treatment system is properly maintained.Gamma-irradiation will successfully inactivate parasites, but is today a politically charged form of treatment, however, ultraviolet irradiation has been chosen as the treatment of choice for drinking water in several large-scale processes in place in several major cities around the world. Conclusions:Parasites remain a constant source of contamination of various crops and drinking water with infectious stages of parasites in sewage and manure.Although soil transmitted helminths have been greatly reduced around the world by the collection and treatment of sewage and the farming of animals in large scale production systems that control manure handling, there remain many places in the world where there remains a fairly direct connection between people and animals and the parasites present in fecal matter. The infections with protists are becoming the more common threat and are known to cross state and national borders on prepared and packaged vegetable crops around the world as part of the globalization of agriculture. Keywords: domestic sewage, control, manure, soil transmitted helminths





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WATER MONITORING METHODOLOGIES FOR CRYPTOSPORIDIUM & GIARDIA/ RESEARCH NEEDS

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Cryptosporidium and Giardia are parasitic protozoa which constitute the leading causes of waterborne enteric disease outbreaks worldwide (Karanis et al. 2007; Baldursson and Karanis, 2011). They infect a wide range of vertebrates, and species within these genera cause human cryptosporidiosis and Giardiasis, which leads to significant morbidity and mortality in both the developing and developed world. Transmission is through the faecal-oral route following direct or indirect contact with the transmissive stages by a variety of mechanisms, including person to person, zoonotic, waterborne, foodborne and airborne (for Cryptosporidium) transmission.Part I: Water detection: We focused on the water methodology developed and applied for Giardia and Cryptosporidium in the last 30 years. Despite a large data has been generated and our knowledge has been substantially improved the question with an effective methodology remains problematic. Methods on the detection of Giardia and Cryptosporidium in water are currently hampered by the lack of suitable methodology mainly affects the first two steps of the sampling and purification/separation not the detection of the (oo)cysts. Our analysis found key gaps in available water detection methodologies and quality data. In order to fill the data and analysis gaps and to provide technical assistance to any governmental, research and monitoring organization, a comprehensive monitoring strategy for Cryptosporidium and Giardia is needed taken in consideration our long term experiences in the field with a world wide leadership position in the field.PART II: The in vitro axenic development of Cryptosporidium parvum in an axenic culture system: Studies of Cryptosporidium are limited by the inability to generate the life cycle and mature oocysts in in vitro culture system. The mass production of the parasite in an axenic in vitro culture system will permit reproducible research on all life stages and transitions essential to clear understanding of the organism's biology and the key for novel diagnostics, drugs development, vaccine targets and to avoid the use of animal experiments for Cryptosporidium. It will also avoid the use of experimental animal models. The cultivation of the parasite in such conditions is not yet accomplished. Research outcomes confirmed the axenic in vitro development of Cryptosporidium and for the first time described their ultrastructure by Transmission Electron Microscopy (TEM). Further studies are necessary to optimize the system towards development of an in vitro model for mass cultivation of the different life cycle stages of Cryptosporidium species to facilitate its use for future approaches of novel diagnostics, therapeutics, vaccine development incl. diagnostic tests for the water monitoring, avoid the use of animal experiments and to control of this parasite and diseases.

DOUBLE-BLIND RANDOMIZED EFFICACY FIELD TRIAL OF ALUM PRECIPITATED AUTO-CLAVED LEISHMANIA MAJOR (ALUM-ALM) VACCINE MIXED WITH BCG PLUS IMIQUIMOD

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Canine visceral leishmaniasis (CVL) is not only an emerging veterinary concern but also a public health problem in endemic areas. The aim of this study was to assess the efficacy and immunogenicity of two doses of aluminum hydroxide (alum) precipitated Leishmania major (Alum-ALM) mixed with BCG plus imiquimod against CVL. A total of 560 owned dogs were serologically tested and 234 healthy dogs with no clinical signs of CVL, no anti-Leishmania antibodies and leishmanin skin test negative were double-blind randomly injected intradermally with either 0.1 ml of Alum-ALM (200 $\mu$ g protein) mixed with BCG (2 × 106 CFUs) plus imiquimod (121 dogs) or injected with 0.1 ml of normal saline (113 dogs). The follow-up showed there was no side effect associated with the vaccination except one case. Strong skin test conversion were found in vaccinated group (30.3%) compared to control group (6.6%) at 22-24 weeks after the booster (p < 0.001). The sero-conversion was 16.3% (18/110) in vaccinated group and 26.4% (28/106) in control group after two transmission cycles. No statistically difference was seen between the two interventional groups (P=0.095). Relative risk in the vaccine group was 59.6%. The efficacy rate of the vaccine was calculated to be 40.4 %. In conclusion, double injections of alum precipitated ALM mixed with BCG and Imiquimod showed to be safe and could decrease the incidence rate from 26.4 to 16.3%.

Keywords: L. major, vaccine, canine visceral leishmaniasis, BCG, Imiquimod, Alum-ALM, Iran

Keywords: Cryptosporidium, water monitoring, Giardia





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COMPARISON BETWEEN COMBINATION THERAPY OF ORAL TERBINAFINE AND CRYO-THERAPY VERSUS SYSTEMIC MEGLUMINE AN-TIMONIATE AND CRYOTHERAPY IN CUTANE-OUS LEISHMANIASIS: AN OPEN RANDOMIZED CLINICAL TRIAL

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Leishmaniasis is a parasitic infection that may lead to a variety of manifestations. Cutaneous leishmaniasis is highly prevalent In Iran. There are many treatment modalities for cutaneous leishmaniasis. The use of oral Terbinafine in the treatment of cutaneous leishmaniasis has recently been considered. Patients with proven direct smear for cutaneous leishmaniasis were divided randomly in 2 groups of 40 cases. For the first group systemic Glucantime prescribed (IM, 15mg/kg/day) for 3 weeks. For the second group oral Terbinafine as two folds of usual dose in the treatment of fungal diseases prescribed [125 mg/day for body weight (BW) 40 kg] for 4 weeks. Both groups received cryotherapy every 2 weeks for 4 weeks. The patients were followed monthly for 3 months after the treatment. Partial (HR = 0.55, CI 95%= 0.3-1.1) and complete (HR= 0.53, CI 95%= 0.3-0.98) clinical improvement in Terbinafine group was much slower than Glucantime group, although at the end of treatment protocols no significant difference between groups were statistically observed (P=0.27). Considering more convenient suitable route of administration and approximately comparable results, it seems that Terbinafine can be used as an alternative treatment, especially in the case of allergy or resistance to systemic Glucantime and especially in children. Of course, more studies have to be done to verify the results.

Keywords: leishmaniasis, oral terbinafine, systemic glucantime, cryotherapy, Kerman, Iran

# COMPARISON OF RA2-ELISA WITH RKE16 DIP-STICK AND DAT IN DIAGNOSIS OF VISCERAL LEISHMANIASIS IN DOGS OF MESHKINSHAHR, ARDABIL PROVINCE, NORTHWESTERN IRAN

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Visceral Leishmaniasis (VL) is the most severe form of leishmaniasis. Different methods including microscopic examination, culture and inoculation to laboratory animals are used for diagnosis of VL, however serological assays are commonly used for detection of antibody in serum samples with a wide range of specificity and sensitivity. The purpose of this study was to compare three serological methods including rA2-ELISA, rKE16 dipstick and direct agglutination test (DAT) for detection of antibodies against VL antigens in order to reduce cross reactivity and false positive results. The assays utilized 350 serum samples from domestic dogs at random and statistical principle with clinical symptoms, asymptomatic and healthy from rural and urban areas of Meshkin-Shahr district. Additional studies were also done using sera from animals and human harboring other infections for the evaluation of cross-reactivity. Samples were assessed and 11.5% and 88.5% by rKE16, 26.9% and 73.1% by DAT, 49.8% and 50.2% by ELISA were indicated as positive and negative respectively. The sensitivity of three methods among symptomatic dogs was 32.4% (rKE16), 100% (DAT) and 52.9% (ELISA). Conversely, rA2-ELISA was less specific for asymptomatic dogs with 46.5% than DAT with 88.9%. In conclusion, these data recommended that rA2-ELISA may be used in parallel with DAT to detect essentially all infected dogs. Efforts should be made to develop a cheap and reliable serologic test for detection of L. infantum among infected dogs.

Keywords: A2 protein, DAT, Dog, Iran, L. infantum, rA2-ELISA, rKE16, visceral leishmaniasis





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Guilan, Iran; 20-22 May 2015IDENTIFICATION OF GENES INVOLVED IN RE-<br/>SISTANCE TO PENTAVALENT ANTIMONIAL<br/>COMPOUNDS (GLUCANTIME) IN LEISHMANIA<br/>TROPICA CLINICAL ISOLATES BY A CDNA-AFLP<br/>TECHNIQUEWhereas, exp<br/>and signalin<br/>kinase and a<br/>sistant isolat<br/>omolecules

<u>Elham Kazemi-Rad</u><sup>1</sup> ,Mehdi Mohebali<sup>2</sup> , Mansour Heidari<sup>3</sup> , Mohammad Bagher khadem-Erfan<sup>4</sup> ,Homa Hajjaran<sup>5</sup>Ramtin Hadighi<sup>6</sup> , Sassan Rezaie<sup>7</sup> , Ali Khamesipour<sup>8</sup> , Soroor Charedar<sup>9</sup>

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Pentavalent antimonial compounds have been the first line drugs for treatment of all clinical forms of leishmaniasis. Recently the efficacy of antimonials has been decreased by emerging of resistant isolates in several endemic regions. Understanding molecular mechanisms of drug resistance is essential for the identification of appropriate markers to monitor the geographical distribution of drug resistance. In present study, for the first time cDNA-AFLP was used for analyzing of gene expression profile between resistant and sensitive Leishmania tropica isolates to identify potential genes involved in drug resistance. Sensitive and resistant strains were isolated from anthroponotic cutaneous leishmaniasis patients and their drug susceptibility was determined by in-vitro assay. Total RNA was extracted and after cDNA synthesis and digestion with restriction Enzymes, the fragments were ligated with specific adaptors. After amplification, the PCR products were separated on polyacrylamide gel electrophoresis. Differentially expressed bands were extracted and after cloning and sequencing, the identities of target genes were determined. Finally, the expression patterns of genes were confirmed using Real-Time RT-PCR. Through cDNA-AFLP approach, 13 target genes were identified; the association of 10 genes with antimony resistance was reported for the first time. Real-Time RT-PCR results demonstrated significant up regulation of 10 genes involved in drug neutralization, antioxidant defense and apoptosis such as multi drug resistance protein A, quiescin sulfhydryl oxidase, RNA binding protein, ubiquitin in resistant isolate compared to sensitive one.

Whereas, expression of three genes implicated in drug uptake and signaling pathways such as aquaglyceroporine, protein kinase and antisilencing factor 1 were down regulated in resistant isolate.Our findings support the idea that distinct biomolecules might be involved in antimony resistance in *L. tropica* field isolates and remarkably a subset of genes differentially expressed to enhance parasite survival in antimonial exposure. Moreover, genes implicated in drug uptake and neutralization suggested as proper markers for identification and monitoring of resistant isolates in endemic regions.

Keywords: Leishmania tropica, resistance to pentavalent antimonials, cDNA-AFLP, Real-Time RT-PCR





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LEISHMANIASIS: OVERVIEW OF THE GLOBAL EPIDEMIOLOGICAL SITUATION, CONTROL IN-TERVENTIONS AND MAJOR CHALLENGES

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Leishmaniasis is a complex vector-borne disease caused by over 20 species of the protozoan Leishmania parasite transmitted by the bite of infected sandflies. The disease is endemic in over 98 countries at least in one of the three major forms; cutaneous, mucocutaneous or visceral. Surveillance and activity data reported to WHO leishmaniasis control programme by the national control programmes of the member states and the WHO regional offices, the WHO Global Health Observatory, and the WHO (2012) publication on the global incidence estimates of leishmaniasis were reviewed. The annual incidence of leishmaniasis worldwide is estimated around 1.3 million cases; out of which visceral leishmaniasis (VL) worldwide is estimated around 300,000 versus the annual average reported 58,000 cases to WHO. Whereas, the annual estimated cases of cutaneous leishmaniasis is around 1million cases versus the reported 220,000. Six countries, Bangladesh, Brazil, Ethiopia, India, south Sudan and Sudan account for over 90% of the global VL burden. Cutaneous leishmaniasis (CL) is much more widely distributed in the Americas, eastern Mediterranean region, extending from Middle East to western and central Asian countries. Mortality data are very scant and largely derived from health facility records. The reported casefatality rates for VL in 2011 were in Brazil (8.4%), Ethiopia (6%) and South Sudan (5%). Epidemics of VL have also affected many endemic countries in the last 10 years. South Sudan was the most afflicted country in the period 2009 to 2012 where over 28,000 cases and 850 deaths were reported from four states. Nevertheless, leishmaniasis outbreaks have affected other countries including Brazil, Ethiopia, Kenya, Georgia, Spain, Syria etc. Relatively high HIV/VL coinfection rates have been reported from Brazil, Ethiopia and India. In Brazil, the reported coinfection rate increased from 2.5% (2005) to 6.6% (2011). According to recent studies and reports, the coinfection rate in northern Ethiopia is around 18%, and in Bihar state, India around 4.5%. Although, the introduction and scaled up implementation of antiretroviral treatment has helped to reduce incidence of VL and increase survival of coinfected patients in many European countries, coinfection is still a major challenge and cause for high relapse and mortality in the endemic developing countries. Regarding leishmaniasis control, the three endemic countries in south Asia (Bangladesh, India and Nepal) are recording remarkable progress in their VL elimination programme achieving over 65% reduction in the annual incidence of cases. However, in Latin America and eastern Africa, VL remains a major challenge as the number of new cases remained the same or even increased in some countries. There is no significant progress in the control of CL due to lack of appropriate tools for large scale implementation. The WHO/NTD roadmap has outlined the leishmaniasis control/ elimination strategic milestones for the different epidemiological zones of the WHO regions. It has envisaged reducing the incidence of VL to less than one case per 10,000 population per year at sub-district level in south East Asia by 2020. In the other regions targets have been set up to control all forms of the disease. In order to achieve the targets, a concerted effort is required, accessing endemic areas by scaling-up service delivery, enhancing the surveillance, ensuring national ownership and prioritizing the research agenda to address key knowledge gaps.

ISOLATION OF PROTOZOAN PARASITES FROM CUTANEOUS LEISHMANIASIS PATIENTS GE-NETICALLY NOT RELATED TO LEISHMANIA SPP. OR THE INSECT TRYPANOSOMATID

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Upward tendency of Cutaneous Lishmaniasis (CL) and emerging of antimony resistance have provoked a serious health problem in most of endemic areas of Iran particularly in Fars province. Moreover, there are increasing reports especially from south parts of Iran that showed strange parasites obtained from CL patients, which morphologically seem to be belonged to lower trypanosomatids as nonpathogenic to humans. This study, therefore, was designed for improved understanding of these strange organisms through whole genome sequencing and morphological analysis. Ultimately, it was assumed findings could produce appropriate programme for control and prevention of CL in Iran. According to morphological analysis, promastigotes obtained from patients suspected to CL were cultured at 37°C and stained by Giemsa for light microscopy analysis. Then, macrophage-amastigote assay was conducted on these field strains to investigate whether they can infect human macrophages. Due to molecular investigation, a Pulsed Field Gel Electrophoresis (PFGE) was carried out to represent comparative karyotyping profile of these protozoa with other Leishmania spp. For building whole genome sequence, finally, Next Generation Sequencing (NGS) technology has applied for two of these odd isolates. The promastigotes form of isolated organisms was morphologically different from Leishmaniaspp, as they had blunted ended and a denser kinetoplast located closer to the nucleus. In addition, it was verified that the parasites were able to grow at 37°C, which is a requirement to develop in the human body. Their potential to infect human macrophage and transforming to amastigote forms have proved as well. Besides, karyotype analysis by PFGE demonstrated that chromosomal patterns of these organisms were not similar to other Leishmania reference spp or to Crithidia genus. The result of NGS, enough surprisingly, was identified: they are a divergent member of the genus Leishmania, and even far from Crithidia, fasciculata and Leptomonas. Taking the morphological and molecular results together, it clearly presents those unusual cases of CL found is not caused by Leishmania spp. or other common monoxenous trypanosomatid from Iran. So far, the potential implications of these organisms in human infections remain unclear, however, a possible explanation is that it could be a hybrid progeny through genetic exchanges between the two divergence genera, Leishmania and Crithidia but also needs to be completely ascertained. Our intriguing finding put forth certain question that whether some cases of CL in Iran actually are leishmaniasis. In overall, further comprehensive research is requested to confirm our preliminary data and specify various aspects of epidemiology, pathology, and transmission rout of these peculiar organisms.

Keywords: leishmaniasis, epidemic, HIV/VL coinfection

Keywords: cutaneous lishmaniasis, Iran, strange protozoa parasites, NGS





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LABORATORY DIAGNOSTIC METHODS IN MA- CURREN

LARIA

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Microscopy method: preparation of thick and thin smears from finger blood of patient; staining with one of the Romanowsky stain methods such as Giemsa and examining microscopically by welltrained laboratory technicians who are experienced in detecting of different stages of malaria parasites. Microscopy method is considered as essential and method of choice in malaria diagnosis in clinically suspected patients. Serological tests: Indirect fluorescent antibody test (IFAT); Enzym-linked immunosorbent assay (ELIZA), etc. may not detect current infection, but usually determine past experience and are most useful in seroepidemiology of malaria and detection of asymptomatic cases of infection in seropositive cases by molecular techniques. Molecular methods: parasite nucleic acids are detectable by molecular techniques such as polymerase chain reaction (PCR) and Real time PCR. These methods are very sensitive and could be used in detection of the malaria parasite and its species in seropositive cases and find asymptomatic cases in elimination phase of malaria program. Antigen detection: various test kits are available to detect malaria antigens: Histidine-rich protein II (HRPII) in Plasmodium falciparum and Parasite Lactate Dehydrogenase (PLD) in all human Plasmodia by immunochromatography (ICT) method. The ICT test is among the rapid diagnostic tests (RDTs), and enough sensitive and specific. It could be simply used for malaria detection in remote areas where facilities for microscopy method are not available. Some other methods such as QBC, magnetic method and digital image analysis have proposed for laboratory diagnosis of malaria but they are not enough practical and reliable.

CURRENT AND FUTURE SITUATION OF MA-LARIA UNDER LOCAL CLIMATE CHANGE IN IRAN

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Climate change impacts are becoming typically a huge threat to health especially for those from developing countries. Malaria is one of the major diseases linked with changing climate and it is the most important mosquito-borne disease in Iran. Although it has had a decreasing trend in the country during the past few years, autochthonous cases are reported from southeastern parts. It was a trend study based on time-series analysis for prediction the trend of malaria, using climate variable in Iran. In this trend analysis using Mann-Kendall Trend test for declaring past situation of disease (1998-2011), the time-series (non-linear) regression analysis applied for future prediction based on baseline year (2000) under temperature rising. Temperature prediction based on time-series analysis has been carried out for 2015-2030. The Average temperature (as climate variable) and three-time period (2015-2020, 2021-2025 and 2026-2030) were included in the regression models. No other variables such as Socioeconomic status, changing the population size, rainfall and other variables considered in the model. Based on the Mann-Kendall trend test, the distribution of malaria decreased in last 14 years. Temperature will be increased from 0.5-0.7 degree centigrade in long-term in Iran (2015-2030). The total cases of malaria were 17,749 in baseline year (2000) for selected provinces. The most cases were from Sistan-Belochestan (7,179) and Hormozagan (4,461) provinces. The mean annual temperature for 2000 used as main exposure. In 16 provinces, malaria cases will be increased for each 3period. The highest incidence will be occurring in Khuzestan province in third time (2310 cases). Totally, trend of malaria under climate change scenario will be increasing up to 2030 in Iran.Based on world malaria report in 2011, Iran is classified to be in the malaria elimination phase and this disease is expected to be eradicated by 2025. Based on our analysis, effects of short-term temperature fluctuations may have not been previously considered but are central to understanding current malaria transmission and the consequences of climate change.

Keywords: malaria, climate change, temperature, time-Series

Keywords: Malaria, Diagnosis, ELISA





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## MOLECULAR IDENTIFICATION OF BABESIA AND THEILERIA SPECIES IN SHEEP AND VEC-TOR TICKS IN THE MOUNTAINOUS AND TEM-PERATE CASPIAN CLIMATES

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Babesiosis and theileriosis are important tick-borne diseases in sheep. The aim of study was to detect Babesia and Theileria species in sheep and vector ticks in mountainous (Kalat and Dargaz areas) and temperate Caspian (Tonkabon and Ramsar areas) climates. One hundred blood samples and ticks were collected from suspected sheep to piroplasmosis in some flocks. The samples were transported to laboratory in cold condition. First, blood smears were prepared and stained by Geimsa method and then, examined with a light microscope at ×1000 magnitude. DNA were extracted from EDTA blood sample, salivary gland and ovaries of tick samples by MBST kit and then, examined with specific primers of Babesia .spp and Theileria.spp by semi nested-PCR. Piroplasme bodies were observed in 40 (40%) of blood samples in mountains climate and 22 (22%) in temperated caspian climate. From 210 collected ticks, six species were identified as follow: Rhipicephalus turanicus 152 (72.38%), R. bursa 40 (19.04%), Dermacentor rakimensis 12 (5.71%), Hyalomma marginatum 3 (1.42%), D. marginatus 2 (0.95%) and Haemaphysalis 1 (0.5%). Dominate tick species in mountains and temperate caspian climate were R. turanicus and R. bursa, respectively. The results of PCR showed that 9 (9%) and 78(78%) blood samples were infected with Babesia .spp and Theileria, spp, respectively. In addition, Theileria infectoin was detected in 4 (4%) of salivary glands of tick. In second round of Semi-nested PCR, 8 (8%) and 1 (1%) Babesia samples detected B. ovis and B. motasi, respectively. Thus, detection of Theileria species reported as follow: T. ovis 42 (53.84%), T. lestoquardi 34 (43.58%), mixed T. ovis and T. lestoquardi 32 (41.02%). One blood sample revealed mixed infection with B. ovis and T. ovis and another one, B. ovis and T. lestoquardi.Based on the results; R. turanicus may be as vector of T. ovis and T. lestoquardi. The frequency of Babesia ovis, and T. lestoquardi infection in mountainous and temperate caspian climates were the same. The frequency of T. ovis infection in the sheep of mountainous climate was higher than the caspian climate.B. motasi infection was only detected in sheep of caspian climate.

Keywords: Theileria spp, Babesia spp, semi-nested PCR, sheep, ticks, Iran

### ANTI-MALARIAL ACTIVITY OF NANO-CHLOROQUINE AND NANO-ARTESUNATE IN MICE INFECTED WITH PLASMODIUM BERGHEI

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Multiple anti-malarial drug resistant Plasmodium falciparum and the emergence of insecticide resistant Anopheles mosquitoes are causing not only the spread of malaria to new areas but also its re-emergence in areas where it had previously been eliminated. This has prompted research towards the discovery and development of new, safe and affordable antimalarial chemotherapies. The aim of this study was to evaluate the anti-plasmodial activity of nano-chloroquine and nano-artesunate on Plasmodium berghei infection in mice. The mice were injected intraperitoneally with inoculum of  $1 \times$ 106 P. berghei infected erythrocytes on the first day. Three days post infection; the mice were divided into 5 groups of 5 mice per group. The groups were subcutaneously treated with nano-chloroquine and nano-artesunate (50 mg/kg/day) and an equal volume of distilled water was given to the negative control group. The treatment was carried out once daily for 4 days. Murine parasitemia was assessed by microscopic examination of Giemsa-stained thin smears of tail blood to monitor the parasitaemia level. The survival rates were monitored for 23 days, and the number of dead mice was recorded every day. The average percentage suppression of parasitaemia of chloroquine and nano-chloroquine groups on day 4 were 100% and the average percentage suppression of parasitaemia of artesunate and nano-artesunate groups on day 7 were 60% and 98% respectively. However, there was a daily increase in parasitaemia in the negative control group. The results of this study verifies the nano-chloroquine antimalarial activity. It seems that more research is needed for determining the effective dose of nano-chloroquine and nanoartesunate for treatment of mice.

Keywords: nano-chloroquine, nano-artesunate, Plasmodium berghei





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DIAGNOSING MALARIA CASES REFERRED TO THE MALARIA REFERENCE LABORATORY IN TEHRAN UNIVERSITY OF MEDICAL SCIENCE, IRAN

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The number of malaria cases is declining worldwide; however, it remains as a serious health problem. Diagnosing unusual cases is the most important issue to manage the problem. This study designed to describe the number of falciparum and vivax malaria infected patients referred to Malaria Reference Laboratory in Tehran University of Medical Science from 2000 to 2012. A retrospective study was conducted based on the collected questionnaires from each patient referred to the laboratory. Diagnosing results and demographic information for positive cases were analyzed using SPSS software. The proportions of malaria cases caused by P. vivax and P. falciparum at the Malaria Reference Laboratory were compared to data obtained from other reports in Iran during the same period. Problematic cases were evaluated for any difficulties in diagnosis or in clinical signs. Scanning and molecular methods were performed whenever there was an atypical case referred to the laboratory. Some of the samples had various difficulties for diagnosing such as presence of fussed gametocytes and schizonts of Plasmodium falciparum in peripheral blood and CCHF like hemoragic disorders. P. vivax caused a large proportion of the cases (76.1%) in comparison to P. falciparum that included smaller proportion (22.8%) and the rest (1.1%) belonged to mixed infection. Most of the positive cases (69.6%) were belonged to Afghanian people. Men (94.6%) showed more infection than women (5.4%), moreover the most infection (44.5%) was seen at a range of 21-30 years. In the case of existing atypical issues to diagnose, it is needed to perform more precise microscopical examination beyond the current standard conditions. Sometimes molecular method is required to verify the exact agent of the disease.

#### SUSTAINABLE VECTOR MANAGEMENT FOR MALARIA ELIMINATION IN IRAN

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Following many years of significant investment and major decline in malaria cases in the Islamic Republic of Iran, the country is now aiming to eliminate local Plasmodium falciparum transmission by 2016, and to be certified as malaria-free by 2025. In 2013, five percent of the population was living in areas with malaria transmission; about 62 percent of all malaria cases (i.e., 1373) were imported from abroad; and approximately 82 percent of the total reported cases were due to P. vivax, with the remainder due to P. falciparum. Only 14% of the 519 autochthonous cases were due to P. falciparum. The final stage of eliminating the disease, however, is the most challenging and requires increasing investment in disease surveillance as well as increased attention to targeting, effectiveness and quality of transmission control through vector control operations. The latter however is threatened by limited number of insecticides available to the control programme due to increasing level and spread of resistance to common insecticides in malaria vectors of Iran. Failure to take steps to mitigate this threat is likely to have severe consequences, both in terms of an increase in the burden of the disease and failure to achieve elimination as well as indirect economic costs. A national plan for insecticide resistance prevention and management in malaria vectors of Iran (2015-2019) has been developed in consultation with national and international experts to guide the Malaria Elimination Programme of the Centre for Disease Prevention and Control (CDC) of Iran in preserving and regaining the effectiveness of insecticides as an important component of integrated approach to management of malaria vectors and to contribute to the sustainability of vector control interventions for malaria elimination in Iran. The National Plan is also intended to support the implementation of the WHO Regional Commitfor the Eastern Mediterranean tee Resolutions EM/RC52/R.6, on Integrated vector management (IVM) and EM/RC58/R.10 on Managing the use of public health pesticides in the face of the increasing burden of vector-borne diseases, as well as the implementation of the International code of conduct on pesticide management. The strategic objectives and the key actions of the National Plan as well as the role and responsibility of different stakeholders in its implementation will be discussed.

Keywords: malaria, diagnose, P. falciparum, P. vivax, Iran

Keywords: malaria, elimination, Iran





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EVALUATION OF RNC1GRA7 FOR NEOSPORA CANINUM SERODIAGNOSIS IN CATTLE AND BUFFALO BY ENZYME-LINKED ASSAY (ELISA)

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Neospora caninum is an apicomplexan protozoan parasite that infects a wide range of warm-blooded animals and also is an important cause of abortion in cattle. Dense granule protein 7 of Neospora caninum is an immunodominant and reliable protein for detection of neosporosis in cattle. The purpose of this study was to produce a recombinant antigen of Nc1GRA7 to develop ELISA and to compare this procedure with commercial ELISA test. A segment of N. caninum DNA corresponding to GRA7 was amplified, sequenced and cloned into expression vector pMAL-c2X. Expression of this plasmid in E. coli strain TG1 was identified by western blotting. After purification and quantification, A Nc1GRA7 based ELISA was developed to detect antibodies against Neospora in cattle and water buffalo. Antibody titers of 108 and122 sera for cattle and water buffaloes were measured by Nc1GRA7 based on ELISAs as well as IDEXX ELISA kit. Sensitivity and specifity of rNc1GRA7-ELISA in comparison with IDEXX ELISA kit were 90.7 and 97.8 percent for water buffalo and 91.37 and 94 percent for cattle. Seroprevalence of neosporosis in 122 serum samples of water buffaloes by Nc1GRA7-ELISA and IDEXX ELISA kit were 56.55 and 57.37 percent and in 108 serum samples of cattle were 53.70 and 51.85 percent, respectively. There was no significant difference between the results obtained by Nc1GRA7-ELISA and IDEXX ELISA kits (P< 0.05). A strong agreement was between two kits in both cattle and water buffalo. The Kapppa index was 0.85 for cattle and 0.83 for water buffaloes. The results revealed that rGRA7-ELISA is a reliable diagnostic tool to detect specific antibodies against N. caninum under field conditions.

Keywords: Neospora caninum, Nc1GRA7, cloning, expressing, ELISA.

# EXPERIMENTAL LIFE CYCLE OF HYPODERAE-UM CONOIDEUM (BLOCK, 1872) DIEZ, 1909 (TREMATODA: ECHINOSTOMATIDAE) PARA-SITE FROM NORTH OF IRAN

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Human echinostomiasis is an intestinal disease caused by the members of family Echinostomatidae parasites. The aim of present research was to identify echinostomatidae cercariae shed by Lymnaea palustris snails, collected from Mazandaran province in the north of Iran, based on the morphological and morphometrical characteristics of the different stages of experimental parasite life cycle. Echinostomatidae cercariae were collected from Lymnaea palustris (Gastropoda: Lymnaeidae) of the north of Iran. To collect metacercariae, 50 healthy snails were experimentally infected with cercariae (50 cercariae for each). To obtain the adult stage, 9 laboratory animals (3 ducks, 2 rats, 2 mice and 2 quails) were fed with 60 metacercariae for each. To identify parasite, the different stages of worm were examined using light microscope and then the figures were drawn under camera Lucida microscope and measures were determined. On Average 15 metacecariae were obtained from each snail that had been previously exposed with cercariae. Ducks released worm eggs in feces after 10-15 days post-infection. Intestinal worms were collected and identified as Hypoderaeum conoideum on the bases of figures and measures of cephalic collar, the number of collar spine, suckers diameter ratio, testes arrangement, etc.H. conoideum cercariae and adult worm are described here. This is the first report of the different stages of the experimental life cycle of this fluke in Iran.

Keywords: Echinostomatidae, *Hypoderaeum conoideum*, experimental life cycle, *L. palustris*, cercaria, worm, parasite





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 PREVALENCE OF MICROSPORIDIA SPP IN PI MOLECU

# GEONS IN A SOUTH REGION OF IRAN, AHVAZ

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Microsporidia are zoonotic parasites that infect humans and a wide range of animals. The aim of this study was to determine the prevalence of microsporidia spp. in pigeons in Ahvaz. Fecal samples were collected from 312 pigeons that were kept in cages at homes, bird stores, as well as from feral and public parks pigeons in different urban areas of Ahvaz. The samples were suspended in PBS, filtered, centrifuged and then potasium dichromat (2.5%) was added and were kept at +4°C until DNA extraction. Microsporidia was detected using direct observation (Weber's modified trichrome staining) and the species was detected by Multiplex-nested PCR technique using specific primers. To confirm the species, PCR products were sequenced. Out 312 sampes, 74 (23.7%) showed structures compatible with microsporidia spores by staining method. Microsporidial DNA was isolated from 121 fecal samples (38.7%). The results of molecular analysis revealed that 71 (22.7%) of pigeons were colonized with single species including: Enterocytozoon bieneusi and E. intestinalis 29 (9.3%), E. cuniculi 8 (2.6%) and E. hellem 1 (0.3%). In 12 (2%) pigeons more than one type of microsporidian spp. were detected. E. bieneusi co-existed with E. cuniculi in 11 pigeons (3.5%) and with E. hellem in 1 case (0.3%). All ITS sequences from positive samples showed 100% homology with the reference genotypes: E. bieneusi D5 (AB897492), E. intestinalis 21F (AB897500), E. cuniculi Khu12 (AB897506), E. hellem Ah102 (AB897504).Findings of present study showed that microsporidia spp. are widely distributed in pigeons in Ahvaz and represents a public health concern that emphasizes the necessity for further precautions to limit the transmission.

Keywords: Microsporidia, pigeons, sequencing, zoonotic parasites

# MOLECULAR IDENTIFICATION OF HEPATO-ZOON SPECIES IN DOGS FROM THE CENTRAL PART OF TURKEY

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Canine Hepatozoonosis is a tick-borne protozoal disease caused by Hepatozoon spp. Two species of Hepatozoon are currently known to infect dogs as Hepatozoon canis and H. americanum. We aimed with this study to determine the frequency of infection with Hepatozoon spp. in stray dogs in central Anatolia region of Turkey. A total of 221 blood samples collected over three year period were evaluated by using genus specific Polymerase Chain Reaction (PCR) designed to amplify a fragment of 666 bp located in 18 S rRNA gene of Hepatozoon spp. Eight (3.61%) blood samples were positive for Hepatozoon spp. Positive PCR products was purified with a PCR purification kit and sequenced. Sequencing results of eight representative amplicons indicated that 6 were 98-99% identical to the sequence of *H. canis* and the other 2 sequences were 95-97% identical to the sequence of Hepatozoon spp. So it was named as Hepatozoon sp. MF. A phylogenetic tree was constructed from the sequences of the tick-borne agents identified previous and in this study using the neighbour-joining method. The nucleotide sequences were compared to the H. canis sequences reported in Turkey using the nucleotide Basic Local Alignment Search Tool (BLAST) program. The results of this study have an importance in terms of the presence of a novel canine Hepatozoon genotype.

Keywords: Hepatozoon, PCR, sequence, canine, Turkey





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 , ZINC AND
 TEN TIPS FOR AUTHORS OF SCIENTIFIC ARTI 

HEAVY METALS (LEAD, CADMIUM, ZINC AND CHROMIUM) CONCENTRATION IN CORYNO-SOMA CAPSICUM (ACANTHOCEPHALAN) ISO-LATED FROM GASTEROSTEUS ACULEATUS FISH IN CASPIAN SEA AND COMPARISON WITH FISH TISSUES

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Over the last decade, the water resources pollution with heavy metals has been described as one of the most important problems in ecology and life of creatures particularly human and animals. However, to assess metals pollution of aquatic ecosystem some indicator such as fishes and birds tissues has been recruited. Hence, the aim of this study was to evaluate the heavy metals (lead, Cadmium, zinc and chromium) concentration in corynosoma capsicum isolated from Gasterosteus aculeatus (G.a) fishes in Caspian Sea and comparison with fish tissues. This experimental study was performed on 250 G. aculeatus captured randomly from several Babolsar fish stations. To determine biometric indexes, fishes and isolated acanthocephalan of this fishes and the parasites were fixed in the Ethanol 70. In the next stage all sample (fish tissue and Acantocephalan parasites) were examined using tissue digestion method. After that, heavy metals concentration and standard solutions were analyzed by atomic absorption system. Data were analyzed using T-test and ANOVA test. The results of this study showed that of 250 G. aculeatus, 80 (32%) were infected to one acantocephalan by minimal rate. Based on key of acanthocephalan identification the genus and species of parasites was corynosoma capsicum. Our investigation showed that the concentration of cadmium metal in dry weight of fishes was more than the amounts reported by world standard references. Also all metals concentration in tissues of non-infected fish were more than tissues of fish infected to one parasite. Our findings confirm that accumulation of metals in tissue has reverse correlation with infection intensity. Moreover, the concentration of cadmium metal was more than world standards. Also based on our results, corynosoma capsicum could be considered as a bioindicator for assessment of the Seawater and other water resources pollution.

Keywords: Acanthocephalan, Corynosoma capsicum, Gasterosteus aculeatus, heavy metals

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Writing a good quality scientific article takes experience and skill. I propose 'Ten Tips' that may help to improve the quality of manuscripts for scholarly journals. It is advisable to draft first version of manuscript and revise it repeatedly for consistency and accuracy of the writing. During the drafting and revising the following tips can be considered: 1) focus on design to have proper content, conclusion, points compliant with scope of the target journal, appropriate authors and contributors list, and relevant references from widely visible sources; 2) format the manuscript in accordance with instructions to authors of the target journal; 3) ensure consistency and logical flow of ideas and scientific facts; 4) provide scientific confidence; 5) make your story interesting for your readers; 6) write up short, simple and attractive sentences; 7) bear in mind that properly composed and reflective titles increase chances of attracting more readers; 8) do not forget that well-structured and readable abstracts improve citability of your publications; 9) when revising adhere to the rule of 'First and Last' - open your text with topic paragraph and close it with resolution paragraph; 10) use connecting words linking sentences within a paragraph by repeating relevant keywords.

Keywords: scientific articles, Ten Tips, scientific writing





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MOLECULAR DETECTION OF NEOSPORA CANINUM IN BRAIN AND HEART SAMPLES OF SLAUGHTERED SHEEP BY NESTED-PCR IN TEHRAN, IRAN

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Neospora caninum is an apicomplexan parasite that morphologically is similar to *T. gondii* and infects a wide range of animal hosts. There is little information about molecular frequency of *N. caninum* in sheep in Iran. The aim of this study was determination of molecular frequency of *N. caninum* in slaughtered sheep in Tehran, Iran. During 2013–2014, 330 samples from slaughtered sheep (180 hearts and 150 brains) were purchased from Vavan abattoir. After DNA extraction, Nested-PCR was performed based on the Nc-5 region of *N. caninum*. *N. caninum* was detected in 6.7% (12/180) and 0.7% (1/150) of heart and brain samples respectively. These findings provide important epidemiological information about *N. caninum* in sheep in Iran. In addition, more researches are needed to clarify the role of *N. caninum* in pathogenesis and its possible economic importantance in sheep in Iran.

Keywords: Neospora caninum, sheep, brain, heart, Nested-PCR, Iran

# PATHOPHYSIOLOGY OF MARSHALLAGIA MAR-SHALLI IN EXPERIMENTALLY INFECTED LAMB

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Species of Marshallagia are abomasal parasites in free-ranging and domesticated ungulates in temperate climatic zones throughout the world. Pervasiveness of these nematodes is significant in various parts of the world. There have been limited researches in the area of Marshallagia marshalli pathogenesis. The aim of this study was to investigate the effects of M. marshalli on the acid secretory capacity of the abomasal mucosa, serum gastrin and pepsinogen concentration and the morphological changes due to parasitic migration to different parts of abomasal tissue in sheep. Ten lambs, approximately around 6 months old, were allotted to two groups of five (A and B). Antihelminth treatment was done to ensure the absence of contamination with worm lambs. The sheep from group A were infected intraruminally with a dose of 5000 third stage larvae (L3) of M. marshalli whereas the sheep of group B were not infected. Fecal samples were collected daily post-infection, Jugular blood samples and abomasal fluid were collected from each lamb twice and once a weak, respectively. Lambs were slaughtered after 30 days and the number of adult worms in the abomasum was counted. Abomasal tissues were sampled for histological examination.By day 21 of post-infection patency was observed in all lambs, and the average number of eggs appeared in feces was three. The average number of EPG reached up to 61.6±35.72 per day by day 30. Dramatic changes in abomasal pH and serum pepsinogen concentrations were seen in larval infected lambs. The serum pepsinogen concentration and abomasal pH were significantly increased in infected group (P<0.001). In all treated lambs, abomasal pH and plasma pepsinogen elevated on day 7 and reached its maximal value on day 16 during post-infection. Abomasal pH and serum pepsinogen began to decrease after day 16 though above normal. The results showed that all infected lambs had mucosal lesions with different densities. Both acute and chronic lesions of abomasal tissue section were seen in microscopic observation. There were not significantly differences in lambs' body weight, eosinophil and gastrin level between control and treatment group.It can be stated that the development of M. marshalli in the abomasal glands of ruminants causes pathophysiological changes, which include a reduced acidity of the abomasal contents, increased abomasal pH, and increased serum pepsinogen concentrations. The reduced acid secretion is explained by a replacement of functional parietal cells by undifferentiated cells. Histology changes include mucosal cell hyperplasia, loss of parietal cells, and inflammatory cell infiltration, which includes numerous granulocytes and lymphocytes. In our study, M. marshalli produced pathophysiological changes following infections. The result of our study is similar to the result in previous experiments with transfer of Teladorsagia circumcincta to non-infected animals resulting in rapid changes in the abomasal function.

Keywords: Marshallagia marshalli, pathogenesis, experimental infections





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EVOLUTION OF FASCIOLIASIS IN THE RUMI-NANT PREDOMESTICATION TIMES AND THE LIVESTOCK POSTDOMESTICATION PERIOD

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Fascioliasis is a food-borne and water-borne zoonotic disease caused by the two trematode species F. hepatica and F. gigantica parasitizing herbivore mammals and transmitted by freshwater lymnaeid snail vectors worldwide. The origins and geographical spread of these two fasciolid digenean species in both the ruminant predomestication times and the livestock postdomestication period are analysed. The ancestor may be found in an ancient fasciolid form infecting old Artiodactyla in Africa during the early Oligocene when the first pecoran radiation occurred. The origin of F. gigantica was probably the result of an adaptation of this ancient fasciolid to bovids, such as ancestors of Alcelaphinae, Reduncinae and Bovinae, during the second pecoran episode, resulting in an explosive radiation during the early Miocene. This origin was probably in the warm, eastern Africa, where the lymnaeid snail Radix natalensis assured the transmission. The origin of F. hepatica was probably in the Eurasian Near East, as a derivation from the same ancient fasciolid or a F. gigantica-close old form introduced with ruminants from Africa during a major sea level lowering in the early Miocene. The origin of F. hepatica is likely the result of colonization of and subsequent adaptation to a new, more northern and temperate-colder region, as well as the result of two host capture phenomena to smaller lymnaeid species of another lineage such as Galba and to midsized ruminants. Paleontological, archeological and historical records, together with genetic data on recent dispersal of livestock species are considered to establish an evolutionary framework for the fasciolids across all continents. A solid molecular epidemiology baseline will help greatly in designing global control measures and local interventions. This evolutionary framework furnishes a new baseline from which to interpret the results of modern genetic techniques applied to the fasciolid parasites and lymnaeid snailo vectors from different regions of the world.

Keywords: fascioliasis, evolution, predomestication time, postdomestication time

PALEOPARASITOLOGICAL FINDINGS WITH SPECIFIC REGARDS TO THE RECENT STUDIES IN IRAN

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Since decades ago, the time of the emergence of paleoparasitology on the scene of the science, several parasitic infections have been identified in different archeological sites worldwide. Ascaris lumbricoides, Trichuris trichiura, Enterobius vermicularis, Dicrocoelium sp. Taenia saginata from beef and Taenia solium from pork as well as many other parasite particles and Schistosoma haematobium eggs in a mummy are among those retrieving helminth eggs in ancient biological remains. Protozoan parasites including Plasmodium falciparum, Leishmania sp. and Entamoeba histolytica have been also identified in archeological sites using ELISA and ancient DNA extraction techniques. Interests among parasitologists and archeologists in Iran are very recently raised in research centers and universities, owing to Chehrabad Salt mummy & Salt Mine Exploration Project northwestern the country and the Burnt city archeological site in the eastern Iran. Paleoparasitological findings from rodent coprolites dated at 500 CE Sassanid Era was recently well illustrated and published. Meanwhile several burial soil samples of pre-historic time in southwest and western parts of the country have been studied in occasions. A comprehensive paleoparasitological investigation is also currently being implemented in the archeological site of Shahre - Sookhteh, 3200 BC in the east of Iran, aiming to understand the status of parasitic infection on that time.

Keywords: paleoparasitology, archeological sites, Iran





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#### MEAT AND FISH BORNE PARASITOSIS : LESSONS FROM ANCIENT MUMMIES

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The description by Ruffer in 1910 of Schistosoma eggs in an ancient Egyptian mummy was the beginning of numerous studies on the parasitological diseases affecting ancient populations. I was somehow involved in the topic when I read, "Is atherosclerosis fundamental to human aging? Lessons from ancient mummies" (Clarke et al, 2014). This paper also mentioned the parasitological results obtained after the autopsy of the famous Nakht mummy and publish again the picture of a Trichinella cyst. This unique picture has led to the popular idea that trichinellosis was present in ancient Egypt as mentioned in nearly all reviews and PhD dissertation dealing with Trichinella. However, there is no convincing evidence that this cyst could be due to Trichinella as no worm remains are visible inside it (Dupouy-Camet, 2014). As discussed in the original paper, the presence of Taenia eggs in the intestinal lumen of the mummy could support the diagnosis of cysticercosis. The use of fluorescent antibodies, recently allowed the identification of cysticercosis in an Egyptian mummy. Other Trichinella reports in ancient mummies were searched to prepare this conference and this parasite was noticed twice: in the Cerro El Plomo mummy, a 8-9 years old Inca child of the XVIth century and in a member of the Inuit accidentally frozen family of Utgiagvik, Alaska. In both occurrences, the images were not very convincing even after the use of indirect immunofluorescence for the Inca mummy. Diagnosing Trichinella in mummies seems very difficult as this parasite is living in muscular cysts surrounded by a collagen capsule and could probably not support the taphonomic processes of mummification. The use of fluorescent antibodies or aDNA amplification could prove the infection. On the opposite eggs of meat or fish borne parasites are easily preserved in mummified tissues and have been reported from different historical times in several parts of the world. Diphyllobothrium eggs have been described in the intestinal content of the 5000 years old Chinchorro mummies from Chile and the prevalence of the parasitosis was estimated to be around 19% at that time in these people living on seafood products. Clonorchis sinensis, Paragonimus westermani, Metagonimus yokogawai & Gymnophalloides seoi have been found in XVIth century mummies of the Joseon dynasty in Korea. In 1975, an extremely well-preserved corpse was found in Jiangling County Hubei, China); according to written records among offerings, the man was buried in 167 BC. Intact eggs of Taenia and C. sinensis were found and the Clonorchis DNA compared with modern Clonorchis eggs showed slight variations. As mentioned above, Taenia eggs were identified in Egyptian mummies and from the mummified corpses of Chehrabad salt mines in Iran. New Generation Sequencing on mummies will certainly allow the identification of meat borne parasites such as Toxoplasma as recently evidenced in an in Egyptian mummy. Finally, further discoveries could be difficult as scientists are now reluctant to perform destructive autopsies. In addition, ethical issues can be discussed as highlighted by Kauman & Ruhli (2010) in their stimulating paper "Without informed consent? Ethic and ancient mummy research".

Keywords: food-borne parasitoses, paleoparasitology, ancient mummies

COMPARISON OF THE PROTEOME PROFILING OF LEISHMANIA TROPICA, L. MAJOR AND L. IN-FANTUM BY TWO- DIMENSIONAL ELECTRO-PHORESIS (2-DE) AND MASS-SPECTROMETRY IN SPECIES CAUSING CUTANEOUS AND VIS-CERAL LEISHMANIASIS IN IRAN

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The mechanisms of virulence and species differences of Leishmania parasites are under the influence of gene expression regulations at posttranscriptional stages. In Iran, L. major and L. tropica are known as the causing agents of cutaneous leishmaniasis, while L. infantum causes visceral leishmaniasis. As a preliminary study, we compared the proteome mapping of the abovementioned three Leishmania species through the 2-dimension electrophoresis, and identified the prominent proteins by mass spectrometry. We reproducibly detected about 700 protein spots in each species by using the Melanie software. Totally, 264 proteins exhibited significant changes among 3 species. 49 protein spots identified in both L. tropica and L. major were similar in position in the gel, whereas only 35 of L. major proteins and 10 of L. tropica proteins were matched with those of L. infantum. Having identified 24 proteins in the three species, we sought to provide possible explanations for their differential expression patterns and discussed their relevance to cell biology. The comparison of proteome profiling pattern of the 3 species identified limit up and limit down regulated or absent /present proteins. In addition, the LC-MS data analysis showed that most of the protein spots with differential abundance in the 3 species are involved in cell motility and cytoskeleton, cell signaling and vesicular trafficking, intracellular survival/proteolysis, oxidative stress defense, protein synthesis, protein ubiquitination/proteolysis, and stress related proteins. Differentially proteins distributed among the species maybe implicated in host pathogenicity interactions and parasite tropism to cutaneous or visceral tissue macrophages.

Keywords: proteome, 2-DE, LC-mass spectrometry, Leishmania tropica, L. major, L. infantum, Iran





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THE HUMAN PROTEOME PROJECT: HUMAN Y CHRMOSOME PROTEOME PROJECT: 2014 UP-DATE

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The human genome project has generated a blueprint for the approximately 20,000 gene-encoded proteins potentially active in any of 230 cell types that make up the human body. However, a substantial number of these genes currently lack any experimental evidence at the protein level. Furthermore, for many genes, there is very little information related to protein function, abundance, subcellular localization, and interactions. The Chromosome- Centric Human Proteome Project (C-HPP) has been intiated to map the entire human proteome in a systematic effort and to enhance our understanding of human biology at the cellular level and lay a foundation for development of diagnostic, prognostic, therapeutic, and preventive medical applications. In Iran, the current efforts are focused on mapping the proteome of human chromosome Y, a unique chromosome, which is in the haploid state and full of repeated sequences but responsible for important biological roles such as sex determination, male fertility, and cancer. Within this project, specific antibodies to human Y chromosome target proteins have been produced using a method involving the cloning and protein expression of protein epitope signature tags. The antibodies are being validated and used to study expression profiles in target cell and in diseased and healthy samples. Owing to the integration of proteomic, genomic, transcriptomic, phenotypic and pathological data, novel findings are emerging from our studies. To address the lack of quality observations of given proteins due to absence of expression in a given tissue, very low abundance, and expression only in rare samples, we are utilizing cell-based approach to analyze the expression of Y chromosome proteins. We observed that several proteins previously known as testis specific were highly upregulated during differentiation of human embryonic stem cells or NT2 cells during differentiation. We also present data showing that suppression of MSY gene combined with shotgun quantitative proteomic approach of resulting cells can enhance our insights about MSY gene function. Our results highlight the importance of utilizing various approaches to enhance the quality of observations for Y chromosome proteins and to increase our understanding about their eminent biological roles in specific target cells.

**Keywords:** roteome, human, chromosome Y

PROTEIN INTERACTION ANALYSIS IN FUNC-TIONAL PROTEOMICS USING SPLIT LUCIFER-ASE COMPLEMENTARY ASSAY

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There are few techniques, which enable analysis of protein interactions independent of gel electrophoresis in functional proteomics. Analysis of protein-protein interactions have been used in many investigation including drug screening, biosensors and basic molecular studies. Firefly luciferase with high quantum yield the ability of different color emissions and suitable structural properties is widely used for design of cell-based biosensors. To develop intracellular reporter, we have employed circular permutation and split firefly reporter strategy wherein a firefly luciferase is genetically split into two non-functional fragments so that luciferase activity is effectively abolished and then these fragments are fused to potentially interacting protein partners and upon induction if the target proteins interact, the fragments of the reporter protein are brought within proximity leading to signal generation that can be measured. For studying a specific signaling pathway, various optional interacting proteins can be taken into consideration in signaling cascades. In one of these approaches, luciferase fragments can be attached to each monomeric unit of a dimer receptor, in which ligand binding induces the dimerization of receptor. Luciferase fragments act more efficiently when they fuse to monomeric unit of a heterodimer, since in homodimer receptors two identical luciferase fragments (either both N or C) may place in right orientation. Based on this strategy, a novel whole-cell recombinant biosensor is designed to detect early-stages of apoptosis and release of IP3. Apoptosome formation is the main step in progress of intrinsic pathway of apoptosis triggered by release of cytochrome c from mitochondria followed by oligomerization of Apaf-1 monomers. In spite of significant experimental support for apoptosome formation, but its detail structure within living cells is not clearly known. In order to direct confirmation of this model and also earlier detection of apoptosis, a novel method using a split luciferase biosensor is designed based on oligomerization of N-luc-Apaf-1 and C-luc-Apaf-1 monomers. Inositol-1,4,5-trisphosphate (IP3) is a crucial second messenger that regulates complicated signaling processes in various physiological events. According to the result, the screening time was very fast and maximum response was obtained up to 11-fold higher than untreated cells. Moreover, the designed biosensor was able to monitor release of IP3 upon induction by different inducers like Bradykinin and ATP. The current biosensor not only provides a specific IP3 detector in vitro but also facilitates monitoring of the response of IP3 in living organisms.

Keywords: proteomics, protein interactions, luciferase





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## MORPHOLOGICAL AND GENETICAL VARIABIL-ITY OF TRICHOBILHARZIA REGENTI FROM NATURALLY INFECTED HOSTS IN MAZANDA-RAN, NORTH OF IRAN

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Trichobilharzia is the largest genus within the family of Schistosomatidae, covering over 40 species of avian parasites. Cercarias of this genus are the main causative agent of cercarial dermatitis. Cercarial dermatitis is known as an endemic parasitic disease in north of Iran, a hypersensitive skin reaction to the penetration of nonhuman schistosome larvae in to human skin. The aim of this study was to determine parasitological and molecular identification of these agents and genomic relationship between them and similar species in other parts of the world. From 2012-2014, we dissected 508 of aquatic birds from Mazandaran province, including, Anseriformes, Gruiformes, Charadriiformes and Phoenicopteriformes. Genomic DNA was isolated by high salt extraction method and ITS region of rDNA were amplified with specific primers ITS-2 Trem, then sequenced area were compared with existing records in GenBank. The morphology of eggs were also measured and compared with the identification key. Nasal schistosomes were found in 45 (8.9%) birds belonged to Anas platyrhynchos and A. clypeata species. Molecular analysis of D2 domain and ITS-2 region of rDNA of-isolated eggs revealed that all samples belong to the Trichobilharzia regenti. Furthermore, sequences of T. regenti isolated from A. platyrhynchos and A. clypeata in the present study were 100% homologous with the haplotype of T. regenti isolated from A. clypeata in France. The measurements also showed that the size of the eggs is nearly identical to the size of the eggs measured in France. Conclusions: Trichobilharzia regenti is the most frequent parasite of aquatic birds in north of Iran. Given the prevalence of T. regenti and its final hosts in the Mazandaran province, it is likely that the origin of this haplotype is this area in north of Iran.

Keywords: Trichobolharzia regenti, cercarial dermatisis, aquatic birds, Iran

#### URINARY SCHISTOSOMIASIS IN IRAN: SUC-CESSES ACHIEVED AND NEW SURVEILLANCE PLAN

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Urinary Schistosomiasis was, until recently one of the major public health problems in Iran. Khuzestan province in the southwestern part of the country with the seven hottest spot regions has been known as the only endemic province in the country. In 1970s infected individuals were estimated about, 40,000-50,000. A long-term study of bilharziasis in Iran in the collection of information has clarified the different bioecological aspects of B. truncatus influencing the previous epidemics in the area. During an intensive investigation in the epidemic periods, neighboring provinces was seen clean from the infection. Soon after in 1968, national control program was started in the country. In the program activities including case finding, treatment, snail control, sanitary measures and health education have been carefully persuaded. The prevalence of the infection has been significantly reduced over the years since the initial control activities were performed. Successful control program besides the improvement of infra structures, mainly known as social determinants of health (SDH) in Iran during the years of epidemics in decades ago till present time have fundamentally changed the scenario.In the country, thanks to all aspects following the elimination target, there have been no new cases of urinary schistosomiasis reported for more than 14 years. Eco biological potentials in the area, besides the present challenges have persuaded the health programmers to modify and consider an up to date surveillance program in the country.

Keywords: urinary schistosomiasis, control, Iran





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MOVING TOWARDS ELIMINATION OF SCHIS-TOSOMIASIS: DESIGN AND IMPLEMENTATION OF AN INTEGRATED MULTIDISCIPLINARY RE-SEARCH PROGRAMME IN ZANZIBAR (UNGUJA AND PEMBA ISLANDS)

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Driven by the 2020 targets set by the World Health Organization and the projected increase in the supply and availability of praziquantel many African countries are stepping up their efforts to control and in some cases eliminate schistosomiasis. In Zanzibar, control of urogenital schistosomiasis has been on going for decades and is now focusing on the possibility of elimination. As part of a larger endeavour (Zanzibar Elimination of Schistosomiasis Transmission), a research study is on going to determine the impact of interventions in addition to chemotherapy, namely behavioural change and snail control. The study is a randomized intervention trial involving 3 different study arms on the islands of Pemba and Unguja. Districts in the first study arm are restricted to biannual mass drug administration (MDA) of praziquantel to the whole at-risk population, the second study arm, includes MDA plus snail control interventions and the third involves MDA plus behaviour change interventions. A total of 45 districts (shehias) are involved on both Unguja and Pemba islands and there are 15 districts in each study arm. Parasites are being isolated for genetic analysis throughout the course of the study. Since the onset of the project in November 2011, four praziquantel treatment rounds have been conducted with a reported coverage of around 80%. On both islands, snail control started in August 2012 and almost 100 natural freshwater bodies in the second study arms are treated regularly with niclosamide when intermediate host snails (Bulinus globosus) are present. Behaviour change interventions were designed together with the communities in the third study arms. Implementation of urinals, teachers packages, safe play for children and laundry areas commenced in October 2012. The results from the 1st and 2nd parasitological surveys will be discussed. A surprising amount of genetic diversity has been observed in Schistosoma haematobium in comparison to isolates from mainland Africa. Conclusions: While progress can be seen at the mid-point of the study, it is clear that there are many challenges for elimination programmes including treatment strategies, community mobilisation, infection hot spots, diagnosis, migration, and suboptimal adherence to drug intake.

Keywords: urogenital schistosomiasis, *S. haematobium*, snail control, behaviour, mass drug administration

# THE NEED FOR SNAIL CONTROL IN THE PUSH TOWARDS SCHISTOSOMIASIS ELIMINATION

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To increase the likelihood that efforts to eliminate human schistosomiasis will be effective and sustainable, means to control schistosome snail hosts (and/or the larval schistosomes within them) will be needed to supplement other control measures like mass drug administration. However, how to control snails or the schistosome sporocysts they harbor? The answer is not an easy one, or snail control would be more widely practiced today. Molluscicidal chemicals surely provide one way forward, particularly when used in focal contexts. We also argue that other means for controlling snails need to be considered, particularly means that exploit natural biological enemies of snails and schistosome sporocysts. Generally, we advocate the use of local biological resources that could be tailored to nearby transmission foci. Among the control agents of particular interest to us in our studies of Schistosoma mansoni transmission in Kenya are digenetic trematodes that produce larval stages that compete with, or prey upon, schistosome sporocysts in their preferred Biomphalaria snail hosts. Schistosomes co-occur in snails harboring the rediae of amphistome and echinostome rediae less frequently than expected by chance, and snails harboring rediae are refractory to subsequent schistosome exposure. In addition, in at least one case, amphistome development in snails appears to be contingent upon subsequent exposure of snails to schistosomes, which are then prevented from developing further. Other potential snail control agents, and the need for more research to reveal new control agents, will also be discussed. Support provided by NIH R01 AI 101438 and a Gates Grand Challenges Grant.

Keywords: schistosomiasis, elimination, snail control





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SCHISTOSOME DIVERSITY: AN UPDATE

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The Schistosomatidae comprises about 100 species of bloodinhabiting digenetic trematodes with major medical and veterinary significance. Although the family is comprised of 4 subfamilies, one of them, Griphobilharzinae, is represented by only one species from a crocodile that, as shown by molecular studies, is a member of a different family, the Spirorchiidae. This is supported by the recent finding of spirorchiids from Nepalese snails that cluster with Griphobilharzia. As presently known, schistosomes are exclusively parasites of birds and mammals. For Schistosoma, relationships among species have been relatively well defined, including the realization that Orientobilharzia clusters within Schistosoma. Molecular data for schistosomes from elephants (Bivitellobilharzia) confirm this is a monophyletic genus consisting of two species, one of which is also reported from the Asian rhinoceros. New collections of mammalian schistosomes from Nepal extend the range of all three members of the S. indicum species group to that country, and highlight the importance of previously undetected genetic variation in the snail host Indoplanorbis exutus with respect to schistosome transmission. Our studies indicate that the diversity of avian schistosomes, that collectively cause swimmer's itch, is considerable. Even in relatively arid New Mexico, 10 species of avian schistosomes have been recovered. Increasingly, molecular signatures can be provided enabling the species responsible for dermatitis outbreaks to be identified. Two new genera of avian schistosomes have recently been erected, and it is likely additional genera await further study as we have found both cercariae and adult worms that do not match any known schistosome sequences. The derived clade of avian schistosomes reveals evidence of extensive host switching, particularly with respect to the snail host. Additional studies are needed to gain a more complete overall understanding of schistosome diversity before it is lost, and to resolve better the deeper phylogenetic relationships among schistosome genera.

Keywords: schistosome diversity, cercariae, mammalian svhistosome

# CERCARIAL DERMATITIS AND THE DIVERSITY OF THE GENUS TRICHOBILHARZIA

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Cercarial dermatitis is an allergic reaction caused by both mammalian and avian larval schistosomes after they penetrate the skin of a mammal or bird. Humans can contract cercarial dermatitis around the world when working or recreating in waters that have both the appropriate snail host and the bird or mammal host. While avian schistosomes are responsible for a majority of cases of dermatitis, in some areas of the world, mammalian schistosomes are most prevalent. Recently, cercarial dermatitis was documented as an occupational hazard in many parts of the world, particularly where rice is grown. It was in 1928 in the United States when the causative agent of cercarial dermatitis was first described. Since that time, cercarial dermatitis has been found to occur around the world, except in Antarctica. Furthermore, the last decade has revealed a diversity of avian schistosomes and snails that have been crucial to our understanding of the current and future way in which we can understand the epidemiology of cercarial dermatitis. The genus Trichobilharzia has achieved notoriety as the primary etiological agent for outbreaks of cercarial dermatitis around the world, especially in the temperate latitudes. The genus Trichobilharzia includes about 35 species that use mainly ducks as a definitive host and snails in the families Lymnaeidae and Physidae. This genus is the most speciose and widespread of the family Schistosomatidae. The focus of this presentation will be on discussing the diversity of Trichobilharzia, and possible mechanisms that lead to this diversity and distribution of cercarial dermatitis. It is likely that host ecology and distribution are both important in determining the global distribution and persistence of species of Trichobilharzia and occurrence of cercarial dermatitis.

Keywords: cercarial dermatitis, Trichobilharzia, diversity, schistosomes





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#### HEARTWORM (DIROFILARIA IMMITIS) OF DOGS – A STUDY IN CREATING RESISTANT ISOLATES

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Parasitologists are well aware that helminths become resistant to pharmacologic agents that are used in their treatment and prevention. In the world of veterinary parasitology, the most recent event that was a surprise to many was the development in many parts of the world of resistance of the equine roundworm, Parascaris equorum, to macrocyclic lactones. The concern has long been that similar macrolide resistance might occur with the filarioid nematodes of humans, Onchocerca volvulus, Wuchereria bancrofti, and Brugia malayi, before the completion of the international eradication program and fortunately - it has remained forestalled. Unfortunately, there have now been recovered from the field a number of isolates of Dirofilaria immitis that are capable of infecting and developing in dogs being prescribed doses of the macrocyclic lactones that were once fully protective. This work has involved a number of different laboratories and teams in North America and Europe. The problem was first noticed in the field by US veterinary practitioners and then was seen by the FDA and pharmaceutical companies as they tried to register new products that were not successful at protecting dogs against recent field isolates of the parasites. The work has involved the collection of these isolates, verification that dogs are not protected against these isolates in trials where they have been challenged with isolates when on prevention, and the molecular characterization of markers for their identification. There have now been tests where all the current heartworm products on the market in the United States have failed to protect dogs against challenge with these resistant strains of heartworm. These isolates have been collected in different cities from the southeastern and central United States. New products have had trouble gaining approval in the United States, as they are required to be tested against new field isolates, and they have failed with molecules that were once fully protective. Molecular markers have now been identified that seem capable of identifying these isolates, and there is high probability that these or similar markers will prove useful in monitoring human filarioids for potential genetic changes as related to macrocyclic lactone resistance such that alternative control methods might be strategically applied in the field if such molecular markers are identified in a given area. The prevention of canine heartworm utilized a different method of control than that utilized in human filariasis. This was a logical choice because in dogs, the adult worms, not microfilariae, are the cause of almost all pathology, while especially in onchocerciasis, the majority of disease is caused by microfilariae, not by the adult form of the parasite. In the case of D. immitis, dogs have been protected against infection with adult heartworms through the administration of the product at low doses to inhibit the development of incoming larvae, whereas, in human filariasis, the goal has been to suppress microfilaremias to prevent transmission by flies biting an infected person rather than to prevent individuals from becoming infected after the bite of an infected fly containing third-stage larvae.Now that it has been shown that filarioid nematodes, in spite of the long life cycles and vector transmission, can develop resistance, it is critical that those working in human filariasis understand the potential risk posed to the world's control of these diseases and begin to implement increased monitoring to allow intervention if resistance appears with the human filaria in a given area. Keywords: Heartworm, Dirofilariasis, Dirofilaria immitis, Filariasis

ANTIBODY TEST OF TOXOCARIASIS AMONG HEALTHCARE EXAMINEES IN KOREA

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Toxocara canis is an intestinal nematode affecting dogs, which causes human infection by ingestion of embryonated eggs or larvae. Although the larvae do not develop into adult worms in the human body, they may survive for several years and migrate to various organs, presenting commonly as covert (adult) or common (child) toxocariasis, and visceral larva migrans and less frequently ocular larva migrans. Toxocariasis is inducing eosinophilia in most symptomless cases but previous data are widely fluctuating. In the present study, we investigated toxocariasis ELISA subjecting healthcare examines of the Korea Association of Health Promotion in Seoul and Gyeongsangnam-do. Total 610 adults (310 from Seoul, 300 from Gyeongsangnam-do, 397 men and 213 women, age 22-80 with mean 53.1±12.2 years) were subjected for multiantigen ELISA including crude antigen of Toxocara canis larvae (TCLA). In total, seropositivity of toxocariasis by ELISA was 8.7% (53/610). The rates in Seoul and Gyeongsangnamdo were 6.1% (19/310) and 11.3% (34/300), respectively. Especially, according to the regional analysis, the positive rate in Gyeongsangnam-do (16.4%) was significantly higher (p < 0.001) than in Seoul (6.9%) in eosinophilia group. Moreover, it has been confirmed that eosinophil count from peripheral blood is moderately correlated with absorbance of ELISA with TCLA. Immunoblot analysis on TCLA recognized antigenic proteins of 28-, 30-, 46-, 56- and 70-kDa bands in their reactivity. Taken together, the prevalence of toxocariasis is significantly high among residents with eosinophilia in Gyeongsangnam-do.It is recommended to screen toxocariasis for symptomless eosinophilia subjects in rural areas.

Keywords: toxocariasis, healthcare examinees, Korea





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#### FAMILIAL TRICHOSTRONGYLIASIS OUTBREAK IN GUILAN PROVINCE, NORTHERN IRAN, MIS-DIAGNOSED WITH ACUTE FASCIOLIASIS

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The suitable climatic conditions and high amounts of rainfall, accompanied with traditional herding, expose the livestock to parasitic diseases in Guilan province. These livestock then play a critical role in transmitting parasitic diseases to humans through infecting the environment by their fecal materials, containing ova and larvae. Guilan province has been recognised as the focus of human fascioliasis in Iran and the whole Asia, because of the two largest outbreaks in the world, affecting more than 15000 individuals. Local physicians consider hypereosinophilia as a marker of fascioliasis, especially when accompanied with abdominal pain, fever and allergic manifestations. So, it is frequently confused with other parasitic diseases prevalent in the area. We are reporting an interesting case of such misdiagnosis in one family. Three sisters of the same family, aged 33, 35 and 38 years old, presented at the same time for medical care with a three-week history of abdominal and epigastric pain radiating to their back, mild to severe diarrhoea, urticaria on their hands, chest and back associated with itching, poor appetite, weakness and weight loss. Blood tests revealed hypereosinophilia of 20, 56 and 60%, while anti-Fasciola and anti-Strongyloides antibodies were both negative. The patients reported regular consumption of fresh vegetables, obtained from their home garden fertilized with fresh sheep manure. Three stool samples from each person were examined using formalin-ether and Kato-Katz techniques under a liver-free diet, and were negative for any ova, cyst and larvae. The patients were diagnosed as having acute fascioliasis based on clinical and epidemiological evidence and received 10 mg/kg of Egaten. One month later, they were readmitted without any sign of recovery. Three new stool samples were examined from each patient, under the same conditions and by the same specialist, which surprisingly were positive for Trichostrongylus ova. The patients were treated by combination of the usual doses of mebendazole and albendazole and their fecal materials were collected for 24 hours after therapy. Two species of Trichostrongylus, (T. colubriformis and T. vitrinus) were identified by morphological and molecular techniques. All other three family members, who were subsequently analyzed, also shed Trichostrongylus eggs. Fascioliasis and trichostrongyliasis share many epidemiological and clinical characteristics, and are both prevalent in climates and living conditions similar to those of Guilan Province. This case highlights the need to consider trichostrongyliasis in the differential diagnosis of fascioliasis patients, particularly in the acute phase when the eggs are not present in stool, and whenever there is a history of eating fresh vegetables fertilized with fresh livestock manure, or close contact with herbivorous animals.

Keywords: trichostrongyliasis, Trichostrongylus colubriformis, T. vitrinus, familial outbreak, Iran

GENETIC VARIATION IN THE SEQUENCES OF THE INTERNAL TRANSCRIBED SPACERS (ITS) OF NUCLEAR RIBOSOMAL DNA (RDNA) AMONG AND WITHIN TOXOCARA NEMATODE OF DOGS AND CATS FROM DIFFERENT AREAS IN IRAN

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Toxocara canis and Toxocara cati are ascaridoid nematodes of dogs and cats. The objectives of the present study were to identify the sequence variation of the internal transcribed spacer (ITS) region within and among isolates of T. canis and T. cati from dogs and cats in Iran. Genomic DNA was extracted from 23 isolates of T. canis and 35 isolates of T. cati collected from dogs and cats in different geographical areas of Iran. ITS region in nuclear ribosomal DNA was PCRamplified. The PCR product of 5 isolates of T. canis and 9 isolates of T. cati were sequenced. The sequences were aligned using the BioEdit software and compared with published sequences in GenBank. Phylogenetic analysis was performed using Mega 5.0 software and Maximum likelihood method. The amplicons of about 1000 and 1100 bp were successfully produced for T. canis and T. cati, respectively. Pairwise comparison of the sequences did not show any differences in nucleotide sequences within T. canis isolates. Likewise, sequences of T. cati were identical and exhibiting 100% homology. However, 12% inter-species sequence difference was found between the isolates of T. canis and T. cati.Regarding to no intra-species variation and a significant inter-species variation in ITS region among T. canis and T. cati isolates, it is concluded that the region can be used for identification and differentiation of Toxocara species.

**Keywords:** genetic variation, *Toxocara canis*, *Toxocara cati*, ITS, Iran





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EVALUATION OF NESTED-PCR AND REAL-TIME PCR METHODS FOR DETECTION OF STRONGY-LOIDES STERCORALIS IN STOOL SAMPLES COMPARED TO CONVENTIONAL PARASITO-LOGICAL METHODS

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Strongyloidiasis or parasitic infection caused by intestinal nematode Strongyloides stercoralis may lead to hyperinfection syndrome and disseminated infections. Without early diagnosis and proper treatment, it may even results into death of the patients. To prevent such consequences, incorporation of highly sensitive diagnostic methods is necessary. Therefore, the present study was performed to evaluate Nested-PCR and Real-time PCR methods for detection of S. stercoralis in stool samples compared to conventional parasitological methods. In order to evaluate Nested-PCR and Real-time PCR methods in field condition, 466 fresh stool samples were collected from endemic areas in Iran including Guilan, Mazandaran, Khouzestan and Hormozgan Provinces and also, from patients referred to the Helminthological Laboratory of School of Public Health, Tehran University of Medical Sciences. All these samples were examined by conventional parasitological techniques (formalin-ether concentration and agar plate culture). From all stool samples, DNA was extracted, using modified in-house (IH) method and amplified by Nested-PCR and Real-time PCR methods. Finally, results of parasitological and molecular methods were compared and validation parameters were determined. Using Nested-PCR method, from the total of 466 stool samples, 117 samples (25.1%) were detected as positive for S. stercoralis; among those 32 samples (7.17%) had not been detected by either formalin ether concentration or agar plate culture techniques. Considering the conventional parasitological techniques as diagnostic gold standard, sensitivity and specificity of Nested-PCR method were 100% and 91.6%, respectively. Realtime PCR method detected 88 samples (18.9%) positive for S. stercoralis infection, among which16 samples (4.2%) had not been detected by either formalin ether concentration or agar plate culture techniques. In this study, 29 samples (6.22%) that were positive using Nested-PCR method could not be detected by Real-time PCR. Sensitivity and specificity of Real-time PCR method were 84.7% and 95.8%, respectively. With increasing parasitic load, CT (threshold cycle) value decreased and there was a statistically significant correlation between parasitic load and CT value (P<0.0001).Molecular evaluation techniques, especially Nested-PCR with more sensetivity are more reliable for detection of Strongyloides stercoralis in stool samples than conventional parasitological methods.

**Keywords:** *Strongyloides stercoralis,* Nested-PCR, Real-time PCR, sensitivity, specificity

PRODUCTION OF TOXOCARA CATI TES120 RE-COMBINANT PROTEIN AND EVALUATION OF ITS POTENTIAL FOR SERODIAGNOSIS OF TOX-OCARIASIS

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Human toxocariasis is a cosmopolitan parasitic zoonotic disease caused by the infective larvae of Toxocara canis or T. cati. Diagnosis is mainly based on antibody detection by specific antigens. Although T. canis has been deemed as the causative agent of most toxocariasis cases, the role of T. cati as the etiologic agent should not be overlooked. Thus, the present study was aimed to produce a T. cati recombinant protein (T. cati rTES120) and evaluate its serodiagnostic potential. The DNA sequence encoding TES120 of T. cati was prepared by performing RT-PCR on RNA isolated from T. cati, using primers previously used to clone T. canis TES120. After deducing the T. cati sequence, the E. coli codon optimized sequence was custom-cloned into glutathione S-transferase pGEX-4T-1 expression (GST)-tagged vector (GE Healthcare, USA), then transformed into E. coli BL21 (DE3) host cell. The expressed recombinant protein was affinitypurified using GST-BindTM resin (Novagen, USA). The diagnostic potential of the T. cati rTES120 protein was determined using IgG4 Western blot. The DNA sequence encoding T. cati rTES120 protein was successfully amplified and the corresponding recombinant protein prepared. The IgG4 Western blot showed the diagnostic sensitivity and specificity of T. cati rTES120 for detection of toxocariasis to be 70% and 100%, respectively. An IgG4 assay using rTES120 T. cati showed good diagnostic potential to detect toxocariasis. A combination of rTES120 from T. cati and T. canis sources would be expected to further increase the sensitivity of the test to detect toxocariasis, for patient diagnosis or epidemiological studies.

**Keywords:** *Toxocara cati*, TES120 recombinant antigen (rTES120), serodiagnosis, IgG4 western blot, toxocariasis





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# STRONGYLOIDIASIS PRESENTING AS ULCERA-TIVE COLITIS; A CASE REPORT

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Strongyloidiasis is a disease caused by the soil transmitted nematode, Strongyloides stercoralis, commonly distributed in tropical and subtropical areas of the world. This disease mainly affects the intestines and the symptoms sometimes mimics inflammatory bowel disease. Due to the northern climate, it is important to inform physicians regarding Strongyloidiasis. Patient's introduction: A 44-year-old male patient who complained of occasional diarrhea, sometimes with blood consulted an Internist. He underwent colonoscopy and ulcerative colitis was diagnosed without biopsy. The patient was treated with Mesalamine together with Prednisolone. The patient's symptoms improved within the first two weeks, but after the third week got worse and he referred to a gastroenterologist. The patient then underwent colonoscopy and biopsy was done this time. In the biopsy, Strongyloides stercoralis larvae were observed. Prednisone and Mesalamine were stopped and Ivermectin tablet was administered. Patient's symptoms improved within a week to ten days and one year after treatment patient did not express any complaint.For diagnosis of Strongyloidiasis, colonoscopy views and biopsy are very useful. Especially in the northern regions of the country, before initiating treatment with immunosuppressive drugs in Patients with ulcerative colitis symptoms, this approach could be recommended.

Keywords: Strongyloides stercoralis, strongyloidiasis, ulcerative colitis

ANALYSIS OF THE 16S-LIKE RIBOSOMAL RNA GENE OF TOXOPLASMA GONDII VIRULENT AND AVIRU-LENT STRAINS

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The rRNA gene cluster of Toxoplasma gondii is approximately 7.5 kb in size and comprises 110 copies per haploid tachyzoite genome. Coding regions for the 16S-like RNAs are used as the basis for molecular systematic analysis. In current study, T. gondii strain variation between virulent and avirulent stains was analyzed using the 16S-like rDNA as a target for PCR-RFLP and PCR-SSCP and Sequencing methods. PCR protocol for amplification of the 16S-like rRNA coding regions from T. gondii genomic DNA was established. A single fragment with the size of approximately 1800 bp was obtained for each T. gondii virulent and avirulent strains (RH, RHL, NED). Simultaneously, Plasmodium falciparum DNA and Cryptosporidium DNA were subjected to same PCR experiment. PCR -RFLP method performed using 10 restriction enzymes such as (HinfI, HincII, Sau3A, DdeI, StyI, TaqI, AluI, HhaI, EcoRI, VspI). PCR products digested with HinfI and AluI were used for Single Stranded Conformational Polymorphism (SSCP) method. Three sets of sequencing primers designed and automated sequencing performed. Sequenced data were searched for similarity by FASTA-SEARCH program based on available data in EMBL/Gene bank database. Agaros gel electrophoresis demonstrated the PCR product with a molecular weight of 1.8kb for three T. gondii strains and Cryptosporidium, but no PCR product observed for P. falciparum DNA amplification, suggesting that it may be due to genetic distance between P. falciparum and other Apicomplexa. A distinctive variation observed between T. gondii strains and Cryptosporidium spp. PCR-RFLP pattern, suggesting that there are considerable genetic differences between T. gondii strains and Cryptosporidium spp. However, T. gondii RH, RHL and NED strains demonstrated completely similar PCR-RFLP pattern. PCR product digested with HinfI and AluI restriction enzymes demonstrated an identical PCR-SSCP pattern for T. gondii virulent and avirulent strains. A section of 1300bp of the 16S-like rDNA PCR product from T. gondii RH, RHL and NED strains were sequenced and compared to each other. Minute differences (1.05% - 2.12%) including substitutions, insertions and deletions observed between three strains. The 16S-like rDNA of T. gondii virulent and avirulent strains is a highly conserved genetic element. It seems in terms of evolution T. gondii could be a newly derived species.

Keywords: Toxoplasma gondii, 16S-like rRNA, strain variation





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# Guilan, Iran; 20-22 May 2015 OCULAR TOXOPLASMOSIS: FACTORS OF EVALU OCCURRENCE AND DIAGNOSIS PLASMII

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Ocular toxoplasmosis (OT) is a major cause of posterior uveitis worldwide but its incidence and prevalence are difficult to establish precisely. In 1993, a survey in a French Hospital Service of Ophthalmology showed that OT was seen in less than 1 per thousand outpatients (Dupouy-Camet et al., 1995). In a study performed in Germany, toxoplasmosis accounted for 4.2 % of all cases of uveitis at a referral centre (Jakob et al., 2009). Around 5000 people develop symptomatic OT each year in the United States (Jones & Holland, 2010). Delair et al (2008) analysing French TR cases showed that 23.5% were acquired and 14.6% were congenital. In southern Brazil, TR prevalence was of 0.9% in 1- to 8-year-olds and of 21.3% in > 13 years olds suggesting sequels of postnatal rather than congenital infections (Glasner et al, 1992). Therefore OT can be a complication of both acute acquired and reactivated congenital toxoplasmosis and its severity can be influenced by variation in parasite isolates, parasitic load, route of infection and host-related factors such as immune function, age and pregnancy. Marked differences were seen between congenitally infected Brazilian and European children: Brazilian genotypes appearing more virulent and divergent than the clonal European lineages (Khan et al, 2006; Gilbert et al, 2008). An increased frequency of the HLA-Bw62 antigen was observed in patients with severe TR (Meenken et al, 1995) and congenital TR is associated with polymorphisms in ABC transporters & collagen genes (Jamieson et al, 2008). KO mice models showed that INF-gamma controlled Toxoplasma dissemination & resulting inflammation and that IL10 decreased severe inflammation (Jones et al, 2006). Recent works have also shown that the parasite virulence factor ROP16 was responsible for the parasite load increase and the Th1 and Th17 ocular inflammatory responses (Pfaff et al, 2014). Extensive TR lesions have been described years ago in immuno-suppressed patients (Holland et al, 1988) and susceptibility was associated with high levels of IL-1 and TNF-alpha, whereas resistance is associated with high levels of IL-12 and INF-gamma (Yamamoto et al. 2000). TR patients were more often carriers of an IL10 allele but no link was found with TNF-alpha gene polymorphism (Cordeiro et al., 2008). Diagnosis of OT is usually based on ophthalmological examination and is confirmed by the response to specific treatment, but also by biological assays including local antibody production, PCR and western blot. Talabani et al (2009) evaluated the sensitivities and specificities of a real time PCR targeting the Toxoplasma gondii 529 bp repeated sequence, of the Goldmann-Witmer coefficient and of the immunoblot in 54 patients with atypical uveitis. The combination of the three tests had 82.5% sensitivity. An algorithm for the biological diagnosis of OT is proposed according to the delay of the eve fluid puncture and the onset of the disease to make the best use of the low volume samples which are most of the time available (Dupouy-Camet et al., in "Toxoplasmosis - Recent Advances" Djurković Djaković O. (Ed.),open access InTech 2012).

Keywords: ocular toxoplasmosis, diagnosis, occurrence

Additional information :

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3- Pfaff AW, et al. New clinical and experimental insights into Old World and Neotropical ocular toxoplasmosis Int J Parasitol. 2014; 44:99-107 EVALUATION THE IMMUNOGENICITY OF PLASMID ENCODING GRA4 OF TOXOPLASMA GONDII AND IL12 IN BALB/C MICE

#### <u>Marjaneh Aghdasi</u><sup>1</sup>, Fatemeh Ghafarifar<sup>2</sup>, Abolhossein Dalimi-Asl<sup>2</sup>, Zohreh Sharifi<sup>3</sup>, Fatemeh Forooghi<sup>4</sup>

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Sever and lethal damage of toxoplasmosis infection in immuno compersive patient and fetous indicates the need for effective vaccine against this problem. One method for enhancing the potency of DNA vaccine is employment of adjuvant. IL12 is extremely potent in enhancing cell-mediated immunity of foreign pathogens and is involved in the differentiation of naive T cells into Th1 cells. GRA4 is dense granule antigen secrets from bradyzoite and tachyziote. This study evaluates and compare the efficacy of pcGRA4 in stimulation of the immune response against toxoplasmosis with and without IL12 as adjuvant. Female Balb/c mice were divided into five groups (n=10 in each group) including three control groups (PBS, pcDNA3, pcIL12), and two experimental groups (pcGRA4, pcGRA4+pcIL12). Mice immunized intramusculary three time in days 0, 21 and 42. The mice were bleeding for antibody assay in days 21, 42 and 63. Four weeks after last immunization, 5 mice of each groups were challenged with fatal RH strain of T. gondii and the survival rate was recorded daily. Seven week after last immunization, 5 mice of each groups scarified and lymphocyte of spleen were cultured with TLA then supernatant were collected for cytokine assay. For lymphocyte proliferation assay we used MTT Mice immunized with (pcGRA4) test. and (pcGRA4+pcIL12) indicate a significant high level of IgG and INFy and low level of IL4 ( $p \le 0.05$ ). The mean survival time for experimental and control groups were 12 and 9.3 days, respectively. Our study indicated that immunization with IL12 adjuvant elicit stronger Th1 type cellular immunity than immunization without adjuvant. The results presented here provide a basis for further researches towards the use of multicomponent DNA vaccines combined with cytokine plasmid and adjuvant. Acknowledgments: This study was supported by funds from Tarbiat Modares University.

Keywords: *Toxoplasma gondii*, GRA4 gene, DNAvaccine, adjuvant, IL12.

56





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PREVALENCE OF ENTAMOEBA GINGIVALIS

AND TRICHOMONAS TENAX IN TABRIZ DEN-TAL PATIENTS BY WET MOUNT, STAINING AND CULTURED METHODS

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Microorganisms capable of colonizing in the oral cavity are rare. Entamoeba gingivalis was the first commensal found in the human oral cavity. The high incidence of Entamoeba gingivalis in individuals with periodontal disease, suggests that these protozoa might have an important role in the etiology of this condition. Trichomonas tenax, a commensal flagellated protozoa, inhabits in human oral cavity. This parasite is cosmopolitian and frequently found in patients with poor oral hygiene and advanced periodontal disease. The clinical importance of this microorganism is the related broncho pulmonary infection named pulmonary Trichomoniasis, that is reported mainly in patients with underlying cancers or other lung disease. The aim of this study was to determin the frequency of Trichomonas tenax and Entamoeba gingivalis of patients referring to periodontics ward of Tabriz Dentistry Faculty in 1391. Materials & Methods: This descriptive cross- sectional study was conducted on patients with periodontal disease, referred to Faculty of Dentistry in fall and winter 2013. Prevalence of Trichomonas tenax and Entamoeba gingivalis was assessed by three methods, microscopically, staining, and culture. Participants were examined by dentist and checked using mirror and periodontal probe. Individuals with periodontal disease for additional assessing were being sent to second step. Sampling method was done by inserting 6 sterile papers in to an envelope for 20 seconds. 3 of papers were assessed in the laboratory using 3 separate slides. The first slide was studied microscopically, and second slide by Giemsa staining. The remaining 3 of samples transferred in to closed plastic pipes containing diamond culture. In next step, the pipes were placed in incubator with 37-centigrade temperature, and daily controlled. Direct observation revealed 2 of participants (2%) were infected by Trichomonas tenax and 16 (16%) were infected by Entamoeba gingivalis. 2 individuals were infected by both parasites and in 80 subjects (80%), there was not any evidence of parasitic infections.By direct observation, in most of the samples, Entamoeba gingivalis was detected. By the method of permanent staining, in 9 (9%) participants, Trichomonas tenax and in 19 (19%) Entamoeba gingivalis were detected. 6 participants (6%), were infected by two parasites and in 66 persons (66%), there was not any evidence of infection by the parasites. By the method of permanent staining, in most of samples, Entamoeba gingivalis was detected. By the method of culture, in 13 (13%) participants, Trichomonas tenax and in 22 of cases (22%) Entamoeba gingivalis were detected. Seven participants (7%), were infected by two parasites and in 58 persons (58%), there was not any evidence of parasitic infections. By the method of culture, which is more sensitive than two other methods, there were more positive cases.Results of this study show that, there is a close relation between incidence of oral parasites infection and oral and dental diseases. High prevalence of parasites in individuals with periodontal problems indicates important role of the parasites in this situation. Keywords: Entamoeba gingivalis, Trichomonas tenax, periodontitis

MOLECULAR IDENTIFICATION OF ACAN-THAMOEBA IN STAGNANT SURFACE WATERS OF TABRIZ, 2014

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Free-living Acanthamoeba species commonly found in natural resources such as soil, water and air, induce various diseases mostly in direct contact with water. Thus, the study of stagnant surface waters contamination for the presence of freeliving amoeba seems necessary. Despite the significance and widespread distribution of pathogenic Acanthamoeba species, there has not been any report for contamination in Tabriz waters. Thus, this study was designed for molecular identification of Acanthamoeba in stagnant surface waters of Tabriz. Materials & Method: In this cross-sectional study, 22 water samples were collected from different regions, from May to September 2014. After filtration, samples were cultivated on non-nutritive agar (NNA). Then, using specific primers for 18srRNA, PCR was conducted for all samples. In microscopic examination, the cyst forms were found in 15 samples, but after amplification of the gene by PCR, 37% (8 samples) were confirmed. In order to ensure the results, some of the isolates were sequenced. The findings indicate the contamination of Tabriz stagnant waters with Acanthamoeba, and effective ways to prevent the spread of diseases and human infections are recommended.

Keywords: Acanthamoeba, surface waters, PCR, Tabriz





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PREVALENCE OF INTESTINAL PARASITIC IN-FECTIONS IN DASHTI, BOUSHEHR FORM 2009 TO 2014

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Parasitic infectious diseases are among the most important health problems and harboring the economic consequences for millions of people in many parts of the world, especially in the developing countries. Epidemiological studies on prevalence of the intestinal parasitic diseases are critical to design appropriate control strategies. Therefore in this study, the prevalence of intestinal parasites in patients referred to the health center of Dashti, Boushehr was analysed from 2009 to 2014. In this study, 1661 cases who referred to health care in central laboratory in Dashti, Boushehr during 2009-2014 were investigated for intestinal parasites. Prevalence of the human intestinal parasites, the relationship of the parasitic infection and demographic characteristics such as sex and age and the season of infection were analyzed. The statistical analysis was done by k-sample proportion test using R statistical software (version 3.1.2) with significant level of 0.05. This study showed that intestinal parasitic infection was as followings: Entamoeba histolytica (44.1%), Giardia lamblia (30.3%), Entamoeba coli (16.5%), Chilomastix mesnili (6.8%), Hymenolepis nana (1.1%), Trichomonas hominis (0.8%), and Enterobius vermicularis (0.4%). The high prevalence of the intestinal parasites would be an alarm for the necessity of prevention programs in this area. Of course, it seems that molecular identification is recommended for Entamoeba histolytica.

Keywords: Parasitic infections, prevalence

PREVALENCE AND CHEMOTHERAPY OF BAL-ANTIDIUM COLI INFECTION IN CATTLES IN SANANDAJ, IRAN

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Balantidium coli is a flattened oval protozoan parasite covered with cilia which belongs to the family of Balantidiidae. It commonly infects human, non-human primates (NHPs), cattles, camels, horses, rats, hamster, pigs and dogs, and has a worldwide distribution. Cattles are grazed on open areas of the farms and mixed with livestock belonging to different families. They have free access to polluted water of environment and to garbage discarded on the rivers. Thus, their usual environment is highly unhealthy. A total of 217 cattle were selected randomly according to the age, sex, health status, management system in various seasons of the year from different villages of Sanandaj province. Feces were collected from the rectum, and placed in sterile bottles for transport to the laboratory. Samples not tested immediately were held at 48C for no longer than 3 days. The collected fecal samples were examined by Stoll's ova counting techniques for determining the number of cysts or trophozoites per gram of feces by their characteristic morphological features as described by Soulsby. 99 cattles (45.63%) were found to be infected with B. coli. In adults, prevalence of B. coli infection (51.64%) was relatively higher than calves (28.58%) and young cattle (40.55%) and the prevalence of B. coli infection was rather higher in female (47.32%) than male (38.46). B. coli infection was significantly (p<0.01) higher in poor health cattle (79.54%) than healthy cattle (24.47%). In this study two drugs named metronidazole and oxytetracycline were used for treatment. Animals that were treated with an oxytetracycline showed a remarkable reduction in the cyst count.

Keywords: Balantidium coli, prevalence, cattle, Iran





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#### PREVALENCE OF INTESTINAL PARASITES IN PATIENTS REFERRED TO CENTRAL LABORA-TORY, EAST AZERBAIJAN PROVINCE 2013

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Parasitic diseases are a major health problem in many communities. This study was aimed to investigate the prevalence of intestinal parasites in patients referred to central laboratory, East Azerbaijan province. 27850 stool samples of patients referred for evaluation of intestinal parasites examined using direct microscopy and formalin-ethyl acetate concentration methods. Based on the results 959 cases (3.44%) were infected with intestinal pathogenenic and non-pathogenic parasites. Entamoeba coli infected 358 cases (1.28%), Blastocystis hominis 352 cases (1.26%), Giardia lamblia 220 cases (0.79%), Taenia saginata 10 cases (0.036%), Oxyuris 8 cases (0.029%), Iodamoeba bütschlü 5 cases (0.018%), Endolimax nana 3 cases (0.01%), Hymenolepis nana 2 cases (0.007%), and Trichomonas hominis 1 case (0.005%).Despite increasing health care facilities, the prevalence rate of intestinal parasites is still considerable. For health promotion especially in countrysides and rural areas, health education and implementation of sanitation measures are recommended for the control of parasitic diseases.

Keywords: intestinal parasitic infection, prevalence, Iran

## SOIL CONTAMINATION OF FREE LIVING AMOEBA (ACANTHAMOEBA AND HART-MANELLA) IN NORTH OF IRAN

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Free-living amoebas are very abundant in nature especially in water and soil. Some species of amoeba induce serious and sometimes fatal infections like keratitis in people with normal and suppressed immune systems. Due to high prevalence of amoebic keratitis in Iran, considering the free-living amoeba is important. A total of 96 soil samples from three areas were collected to examine soil contamination with Acanthamoeba spp. Soil samples were investigated for the present of Acanthamoeba spp. using flotation method. The identification of Acanthamoeba at the genus level in this study was based on distinctive features of double walled cysts. From 96 samples that were studied in different environmental locations in Sari, 38(39.6%) contained Acanthamoeba and 5(5.2%) contained Hartmanella. The results of the present study revealed that soil resources of this area were contaminated with opportunistic amoebas such as Acanthamoeba and Hartmanella and this may lead to severe disease in high-risk people, such as immunocompromised patients.

Keywords: Acanthamoeba, Hartmanella, free living amoeba, Iran





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#### ACANTHAMOEBA IN SEA AND HOT SPRINGS WATER SAMPLES FROM MAZANDARAN PROV-INCE

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Introduction & Objectives Acanthamoeba as a free-living amoeba, commonly found in soil, dust and water sources (such as lakes, rivers, hot springs and sea water), it can cause rare, but severe, illness such as Acanthamoeba keratitis and Granulomatous Encephalitis. So this study was designed to detect Acanthamoeba in some surface water in Mazandaran provinc.Fourteen samples were collected from different surface water (rivers, sea water and hot spring) in Mazandaran province. Each samples (500ml) filtrated by membrane filter, then the isolation of the amoebas was performed in nonnutrient agar inoculated with Escherichia coli. Finally the amoebas were identified according to morphological criteria and confirmed by PCR methods. Twelve samples were positive by Culture and PCR methods. Fifteen percent of hot spring samples and all rivers and seawater samples were positive for Acanthamoeba cysts. Although further study is needed to confirm the virulence levels of these amoebae isolates, results of this study highlight a need for more attention to free living amoeba in study area, especially for individuals with immune deficiency situation and contact lens wearers

Keywords: Acanthamoeba, PCR, water, Iran

# ISOLATION OF FREE LIVING AMOEBAE FROM SURFACE WATER IN MASHHAD, IRAN

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The Genus Acanthamoeba has been associated with Granulomatous Amoebic Encephalitis and Amoebic Keratitis. They are distributed worldwide and have been isolated from soil, dust, air and stagnant water. Due to lack of information about Acanthamoeba water pollution in Mashhad, we decided to investigate this issue. From December 2012 to June 2013, 61 samples (500 ml) were collected from squares and ponds of parks in Mashhad, Iran and carried to the laboratory of parasitology of Guilan University of, Medical Science. Water samples were filtered and cultured on Non-Nutrient Agar (NNA) plates. Identification of Acanthamoeba was based on the morphology of cyst on NNA medium. Out of the 61 samples, in 13 samples the free-living amoeba found in culture medium according to morphological criteria. In this study the Acanthamoeba were detected in many of the water ponds of parks and squares in Mashhad. It should be mentioned that, since this city annually receives millions of pilgrims, it is necessary that public health organizations should provide sufficient information for preventing of contamination especially in high risk groups. They should be aware of the possible role of water in the transmission of Acanthamoeba spp. diseases. However, further study is needed in order to confirm the pathogenicity of these amoebae isolates.

Keywords: Acanthamoeba, water, Iran





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### FREQUENCY OF INTESTINAL PARASITIC IN-FECTIONS IN PATIENTS ATTENDING TO MED-ICAL LABORATORY OF SHAHID GHAZI HOSPI-TAL IN SANANDAJ IN 2014

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Parasitic infections are one of the major health problems in developing countries, which are directly related to personal hygiene, therefore, this study aimed to evaluate frequency of intestinal parasitic infections in patients attending to Medical Laboratory of Shahid Ghazi Hospital in Sanandaj in 2014 Samples, were taken from 1,700 patients referred to the laboratory during the spring of 2014. The samples were examined by direct examination with Lugol's iodine and then investigated with optical microscope, the suspected Samples rechecked in parasitological laboratory of Kurdistan University of Medical science and the diagnosis has been approved or revised. 321 out of 1,700 people were infected with parasitic infection, the prevalence of infection was 18.9% and 179 of this infected people were female (10.52%) and 142 male (8.3%), One patient (male) was infected with hematophage form of Entamoeba histolytica (0.06%). A total of 90 patients (60 females and 30 males) were infected with Giardia lamblia cysts (5.3%), 50 patients (18 females and 32 males) were infected with Blastocystis hominis (2.9%), 7 patients (3 women and 4 men) were infected with Iodamoeba butschlii (0.4%), 150 patients (88 female, 62 male) were infected with Entamoeba coli (8.8%), 20 patients (8 female and 12 men) were infected with Endolimax nana (1.8%), 1 patient (male) was infected with Strongyloides stercoralis (0.06%), 1 patient (female) was infected with Taenia saginata (0.06%), 1 patient (male) was infected with Trichostrongylus (0.06%) and the patient was referred to Tehran and repeated test confirmed the diagnosis.Although the collected data did not revealed the prevalence of parasitic disease in the area exactly, high parasitic infections among patients referred to medical laboratory, recruiting expert persons for correct diagnosis of infection and pay more attention to public health can be effective to control and prevent the infection.

Keywords: parasitic infections, intestinal protozoa, intestinal worms, Iran

Differential detection of Entamoeba histolytica from Entamoeba dispar by parasitological and Nested Multiplex Polymerase Chain Reaction methods

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Amebiasis is an intestinal illness caused by a one-celled parasite (amoeba) called Entamoeba (E) histolytica. E. histolytica and E. dispar are morphologically undistinguishable but have genetic and functional differences. E. histolytica is invasive and cause amoebiasis, but E. dispar cause an asymptomatic colonization, which does not need to be medically treated. We have performed a nested multiplex Polymerase Chain Reaction (PCR) targeting small subunit rRNA (Ribosomal ribonucleic acid) gene for differential detection of E. histolytica and E. dispar directly from stool samples. All fecal samples were collected without preservation and screened for amebic cells by parasitological methods. Fecal samples containing amebic cells were stored at -20° C until DNA extraction. DNA extraction was carried out using a DNA extraction kit. The genus specific primers were designed using nucleotide sequences of 18S-rRNA gene of Entamoeba. Thirty one (4.28%) stool samples out of 724 samples were positive for E. histolytica/ E. dispar. The nested multiplex PCR illustrated that the size of diagnostic fragments of PCR products was obviously different for two Entamoeba species, the specific product size for E. histolytica and E. dispar was 439 and 174 bp, respectively. The nested multiplex PCR was positive in 25 out of 31 stool specimens in that 17(54.8%) samples were positive for E dispar and 8 (25.8%) for E. histolytica. Conclusions: Nested multiplex PCR was useful for specific detection of E. histolytica and E. dispar in stool samples. In current study we detected that *E. dispar* was more prevalent in our study area.

**Keywords:** *Entamoeba histolytica*, *Entamoeba dispar*, Nested Multiplex PCR





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# ISOLATION AND IDENTIFICATION OF PATHO-GENIC FREE LIVING AMOEBA FROM SURFACE AND TAP WATER OF SHIRAZ USING MORPHO-LOGICAL AND MOLECULAR METHODS

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An investigation was conducted to determine the presence of free-living amoebae (FLA), especially Acanthamoeba and Hartmanella in waterfronts of parks and squares and tap water of Shiraz, Iran. FLA is considered pathogenic for human. These ubiquitous organisms have been isolated from different environment such as water, soil and air. Eighty-two water samples were collected from different places of Shiraz during summer 2013. All samples were processed in the deptartment of parasitology and mycology, Shiraz University of Medical Sciences. Samples were screened for FLA and identified by morphological characters in the cultures. PCR amplification targeting specific genes for each genus and sequencing determined frequent species and genotypes base on NCBI database. Overall, 48 samples were positive for Acanthamoeba and Hartmanella in non-nutrient agar culture based on morphological characteristics. The PCR examination was done successfully. Sequencing results revealed T4 (62.96%) genotypes as the most common genotype of Acanthamoeba in the Shiraz water sources. In addition T5 (33.33%) and T15 (3.71%) were isolated from water supplies. H. vermiformis was known the dominant species from this genus. The result highlights a need for taking more attention to water supplies in order to prevent illnesses related to free-living amoebae. T4 and T5 genotype of Acanthamoeba have been isolated from clinical samples. Recent reports have shown mixed infection of Hartmanella and Acanthamoeba in AK cases.

Keywords: Acanthamoeba, Hartmanella, surface water, tap water, Iran

# GENERAL INFLAMMATION IN PATIENTS WITH INTESTINAL PROTOZOAN INFECTIONS IN TEHRAN, IRAN

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Protozoa are unicellular eukaryotes. The prevalence of protozoan diseases in human populations is associated with health and sanitation conditions. A type of intestinal protozoan pathogens is the cause of infection and serious injuries in humans with and without clinical symptoms. The purpose of this study was to examine the general inflammation by measuring the rate and ratio of white blood cells in patients with intestinal protozoa. This study was conducted on blood samples of 20 people consisted of 10 control subjects and 10 patients with parasitic infection (average age 21±2 years) referred to medical centers in Tehran. The samples were treated with Wright stain method; and neutrophil to lymphocyte ratio was calculated using WBC diffraction method. According to the results of this study, a significant increase was observed (P <0.05) in neutrophil to lymphocyte ratio in patients with parasitic infections (2.552±0.246) compared to the control group (1.957 $\pm$  0.137).Based on the results of this study, the increase in neutrophil to lymphocyte ratio may indicate the active development of infection and an inconvenient prediction of protozoan infection. On the other hand, neutrophil to lymphocyte ratio can be a potential useful parameters for evaluating the stress system activity in patients infected with the intestinal protozoa.

Keywords: general inflammation, patients, intestinal protozoa





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INTESTINAL PARASITIC INFECTIONS AMONG RURAL INHABITANTS OF HAMADAN, WEST OF IRAN

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Intestinal parasitic infections are among the most important indices indicating the sanitation level and hygiene status of the society. This study aimed to determine the prevalence of the intestinal parasitic infections among rural inhabitant of Hamadan, West of Iran. Totally 228 fecal samples were collected from 50 families of 7 villages that were directly and indirectly involved in activities with livestock and other domestic animals during spring 2012. The demographic variables of the farmers gathered by interviewing, including age, educational level, sex, direct or indirect contact with animals and animal keeping place. Fecal samples were concentrated using formalin-ether sedimentation technique and examined by iodine stained wet mount method. Indistinguishable samples underwent trichrome staining method. Eighty out of 228 (35.1%) samples were diagnosed to be infected by intestinal parasites including Entamoeba coli 43 (18.9%), Blastocystis hominis 32 (14%), Endolimax nana 16 (7%), Iodamoeba butschlii 9 (3.9%), Giardia lamblia 5 (2.2%), Taenia spp. 2 (0.9%), Hymenolepis nana 2 (0.9%), Chilomastix mesnili 1 (0.4%), Trichuris sp. 1 (0.4%) and Entamoeba histolytica/dispar 1 (0.4%). No significant difference was observed considering the infection rate in humans with indirect and direct contact with livestock. Coinfection of E. coli and B. hominis, E. coli and I. butschlii, and also E. nana and G. lamblia were observed that were statistically significant.Based on the results of this study, the prevalence of infections with some intestinal parasites is some how high in the Hamadan area considering that the most of the parasites are non-pathogenic and, generally, pathogenic ones have been reduced comparing to the past. Thus the existence of Taenia spp. and H. nana could not be ignored.

Keywords: parasitic infections, helminth, protozoa, prevalence, Iran

#### PREVALENCE OF INTESTINAL PARASITES AMONG THE ELDERLY IN NURSING HOMES OF LARESTAN IN 2014

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In some cases, reaching perfection and aging is associated with some weaknesses. With aging, the immune system is weakened and the individual becomes prone to infections. One of the major health problems in old age is the risk of intestinal parasitic infections that exacerbates malnutrition in the elderly. Identification and treatment of parasitic infections is an effective step in maintaining and improving the health of the elderly. Materials & Method: In this crosssectional, stool examination (formalin-ether method) was carried out for 89 elderly people and workers in the nursing home. The results were analyzed using descriptive and inferential statistics. The findings of examination showed that 56 subjects (62.9 percent) were infected with various pathogenic and non-pathogenic parasites. Twelve elderly (13.5%) subjects and 4 workers (4.5%) were infected with pathogenic parasites. Seven subjects (7.8%) were infected with Giardia lamblia, 3 (3.3%) with Entamoeba histolytica and 6 (6.7%) with Hymenolepis nana. Amounts of non-pathogenic parasites including E. coli, Chilomastix mesnili and various intestinal Trichomonas were significant. No significant difference was found between the elderly group and the staff in terms of infection with parasites. The presence of intestinal parasites in children and the elderly leads to digestive disorders and malnutrition. In the elderly, decreased appetite and in some cases lack of access to adequate food worsens the situation. Therefore, it is necessary to carry out periodical examination for these people so that appropriate treatment measures to be taken for them to improve their experience of old age.

Keywords: prevalence, intestinal parasites, lderly, Iran





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# HIGH CONTAMINATION OF SOIL SAMPLES TO FREE LIVING AMOEBAE IN SARAB AND MEHRBAN COUNTIES, EAST AZARBAIJAN PROVINCE, IRAN

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Free-living amoebae (FLA) are unicellular protozoan parasites widely spread in a variety of natural habitats, including dust, lakes, rivers, swimming pools, thermal baths, tap water, sources of waters receiving industrial refuse, sewage, as well as fluids or tissue from healthy human oropharynx. Presence of FLA in these environments is a hygienic risk and induces diseases including chronic granulomatous amoebic encephalitis (GAE), granulomatous skin lesions, keratitis and primary amoebic meningoencephalitis (PAM) especially in individuals with immunodeficiency. The main aim of the current study was to identify the presence of FLA in the soil samples of Sarab and Mehrban counties, East Azarbaijan province, Iran. 55 soil samples were selected across the parks of Sarab and Mehrban counties from public places and recreational regions in the last three months of 2014. One hundred gram (100 gr) of soil samples were dissolved in sterile distilled water, remained for about an hour and filtered with filter paper. The filters were cut out and placed on 0.6% Non-nutrient agar (NNA) medium along with Escherichia coli as a food source for amoebae. Identification of the FLA were done using page key. Overall, 39 out of 55 collected samples (70.9%) were positive for free-living amoebas. The positive samples contained Acanthamoeba spp. (71.79), Vahlkampfiids spp. (5.12), Hartmannellidae spp.(7.69) and Thecamoeba (2.56). The morphological characteristics of the identified amoebas consisted of trophozoites with multiple acanthopodia and double-walled cysts with wrinkled external wall, and angular polygonal inner wall in Acanthamoeba, wormy shape trophozoites and round cyst shapes in Vahlkampfiids, limax trophozoite with one small nucleolus and small spherical or ovoid cyst in Hartmannellidae. High percentage of free living amoeba in soil samples is considered a hygienic risk for public health mainly for individuals with immune deficiency and contact lens wearers, therefore health authorities must be alert of FLA presence in such environments and alarming sings should implicated in recreational areas.

Keywords: soil samples, free-living amoebae, *Acanthamoeba*, Vahlkampfiids, Hartmannellidae, *Thecamoeba* 

STUDY OF ACANTHAMOEBA SPP. IN CONTACT LENS SOLUTION

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Acanthamoeba keratitis occurs primarily among users of soft contact lenses. The primary risk factor is use of a multipurpose contact lens solution. So this study was designed to investigate Acanthamoeba spp., in contact lens solution of university students.During 2014-2015, twenty two contact lens solutions collected from university students in Guilan, checked for Acanthamoeba spp. All plates were sealed, incubated at 30°C, and monitored daily for two weeks so that the amoebas grew and changed into cysts. The isolation of the amoebas was performed in non-nutrient agar inoculated with Escherichia coli. The amoebas were identified according to morphological criteria. All samples were negative for Acanthamoeba spp., although some cultures medium were positive for fungi.Negative results for Acanthamoeba spp. may be justified because university students are more aware of higher sanitation in compare to other groupe of society. Culture contamination may be due to growth of fungus in nonnutrient agar. This study could be regarded as a base for other studies to determine prevalence of Acanthamoeba and fungi in contact lens solution. More studies with more samples in other group of society with different education levelsis recommended.

Keywords: Acanthamoeba, contact lens, keratitis, Guilan





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# PRESENCE OF ACANTHAMOEBA SPP. IN SWIM-MING POOL IN RASHT CITY

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Acanthamoeba is a free living amoebae commonly found in the environmental sources such as soil, water and dust. This parasite is the causative agent of amoebic keratitis in people using contact lens and granolomatose encephalitis in people with immune deficiency. Because there is no information about Acanthamoeba in swimming pools in Rasht, and regarding to the role of water in life cycle of this parasite, the present study was designed to investigate presence of Acanthamoeba in water of swimming pool in Rasht. This study was done in autumn 1393; seven samples were collected from swimming pool and transferred to Guilan University of Medical Sciences laboratory to study. For each sample approximately 500 ml water were filtered through 0.45µ membrane filter. Filter was transferred on non nutrient agar plate seeded with E. coli, incubated at 30 degree centigrade and monitored daily for two weeks. The Acanthamoeba amoebae were found in 2 (28%) of water samples of swimming pools. One sample was taken from main swimming pool and another sample belongs to Jacuzzi.Since the pathogenic type of this parasite probably can grow up in warm water and one of the positive samples in present study was taken from Jacuzzi, it is probably a pathogen. So there is potentially the risk of water borne diseases due to Acanthamoeba to people who swim in this kind of waters, although more studies are needed to prove it.

Keywords: Acanthamoeba, swimming pool, encephalitis, keratitis, Rasht IN VITRO AMOEBICIDAL ACTIVITIES OF ZIZIPHUS VULGARIS AND TRIGONELLA FOE-NUM GRAECUM ON ACANTHAMOEBA CASTEL-LANII CYSTS AND TROPHOZOITES

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Amoebic keratitis is difficult to treat without total efficacy in some patients because of cysts, which are less susceptible than trophozoites to the usual treatments. The aim of this study was to evaluate the in vitro amoebicidal activity of the aqueous extracts of Trigonella foenum graecum and Ziziphus vulgaris.Corneal scraping isolated from a keratitis patient. The specimen was inoculated directly onto the surface of 1.5% non-nutrient agar (NNA). Cysts and trophozoites in the resultant suspension were counted, and the suspension was standardized to be 25×104/ml. The aqueous extract of Trigonella foenum graecum in different concentrations (200 mg/ml, 300 mg /ml, 350 mg /ml, 400, mg /ml, 600 mg /ml, 750 mg /ml ) and Ziziphus vulgaris in concentrations of (25 mg/ml, 50 mg /ml, 100 mg /ml, 200 mg /ml, 400 mg /ml, 500mg /ml ) were performed. Acanthamoeba were incubated with the mentioned concentrations for 24-72 h along with 0.02% chlorhexidine and PBS as positive and negative control.Among the extracts tested, Ziziphus vulgaris showed the strongest amoebicidal effect on the trophozoites and cysts. In the presence of 200 mg/ml Ziziphus vulgaris extract, no viable trophozoites and in the 500 mg/ml concentration, no viable cysts were determined in 24th hour. In the case 400 mg/ml concentration of Trigonella foenum graecum, no viable trophozoites and in the 750 mg/ml concentration, no viable cysts were determined in 24th hour. Conclution: The present results revealed that both extracts could be considered as appropriate candidates for treatment of Acanthamoeba infection.

Keywords: Acanthamoeba, aqueous extract, Ziziphus vulgaris, Trigonella foenum graecum, in vitro





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# MORPHOLOGICAL IDENTIFICATION OF FREE-LIVING AMOEBAE FROM HOT SPRINGS IN MA-ZANDARAN PROVINCE, NORTHERN IRAN

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Free living amoebae include many genera such as Acanthamoeba, Naegleria and Hartmannella. Acanthamoeba spp. and Balamuthia mandrillaris are opportunistic free-living amoebae capable of causing granulomatous amoebic encephalitis (GAE) in individuals with compromised immune systems. Due to their thermotolerant nature, these amoebae could be present in water sources and therefore the aim of the present research was to isolate FLA from all hot springs in Mazandaran province by employing morphological method. Sixty-three water samples from twenty-one hot springs were collected from cities of Mazandaran. The samples were filtered through a cellulose nitrate membrane and cultured on nonnutrient agar plates containing E. coli. These plates were followed for 2 months and the morphological criteria were tested using page key. Result: FLA was detected in 60 plates (95.23%) out of 63 water samples in Mazandaran province. Twenty seven plate (42.85%) were identified as Acanthamoeba and Hartmannellidae, 21 plate (33.33%) as Hartmannellidae and Vahlkampfiids and 6 plates (9.52%) scored as Vahlkampfiids and Hartmannella and Acanthamoeba and 9 plates (14.28%) were negative. Acanthamoeba were characterized by a single nucleus, dense nucleolus, and filamentous projections called acanthopodia. Cysts were double walled with star shape or triangular endocysts. Hartmannellidae were detected by their spherical cysts and wormy shape trophozoites by the light microscopy. Vahlkampfilds were determined using their characters including round cysts mainly with double wall shape. The results indicated the presence of potentiallv pathogenic Acanthamoeba, Hartmannellidae and Vahlkampfiids in Hot springs in Mazandaran province, North of Iran. Education of high risk people, improved filtration and posting of alarm signs are necessary to eliminate such amoebae from lot springs.

Keywords: *Aanthamoeba* spp, Vahlkampfiids, *Hartmannella*, hot springs, Mazandaran,

# PREVALENCE OF CRYPTOSPORIDIUM PARA-SITE IN CHILDREN OF LARESTAN IN 2014

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Cryptosporidium parasite is from coccidia group that causes digestive diseases in people who have a weak immunity system or suffer from AIDS. This parasite has no specific host. Although, the infection is usually stopped spontaneously in normal individuals, the quality of self-infection and extension of this parasite is possible for sustaining of the infection. The parasite can produce acute and chronic digestive infections in children. The continuation and intensity of illness can cause much harm in children. It is certainly influential for the health of the society to know about the ill children. In this research, we collected 541 samples of feces from eight area in south of Iran. 64 samples were watery as having diarrhea. We used the staining method (Ziehl-Neelsen's modified by Henriksen) for diagnosis. Staining of the samples didn't show any sign of infection with" Cryptosporidium" in these children. It was probably because either the feces samples were not sufficient or at the time of survey (autumm and winter), the rate of infection had been low.Nevertheless, further studies is necessary to obtain the real prevalence of the disease which is a crucial danger for the infants' health.

Keywords: Cryptosporidium, Children, Iran





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*Guilan, Iran; 20-22 May 2015*DETECTION OF POTENTIALLY PATHOGENIC PREVAL

# FREE-LIVING AMOEBAE IN WATER BODIES IN ILAM PROVINCE

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Free-living amoeba are unicellular protozoan with a wide distribution in environments. Naeglaria fowleri, Balamuthia mandrillaris and Acanthamoeba are some of the FLA species known to be pathogenic in humans. Acanthamoeba spp. are the causative agent of amoebic keratitis (AK) and granulomatous amoebic encephalitis (GAE) while Naegleria fowleri and Balamuthia mandrillaris have been associated with amoebic meningoencephalitis (PAM) and GAE, respectively. The present research aimed to detect Free-living amoeba in recreational water sources and hot spring in Ilam province, using morphological criteria. Thirty four samples were collected, 25 sample from surface waters of recreational water centers including parks and ponds of Ilam and 9 samples collected from hot spring waters of Dehloran city. Each sample was filtered through a cellulose nitrate membrane and cultured on 1% non-nutrient agar along with E. coli. These plates were followed for 4 weeks and the morphological criteria were determined using page key. According to appearance of cyst and trophozoites of 34 samples, 27 (79.4%) were positive for free-living amoebae. Among them 18 samples belonged to Vahlkampfiids and 9 sample were positive for Acanthamoeba. The identification of Acanthamoeba cyst was based on distinctive features of double walled and trophozoits were flat in shape with one nucleus and large karyosoms. Vahlkampfiids were determined by spherical shape cysts with distinctive double walls and wormy shaped trophozoites with large vesicular nucleus. High occurrence of free-living amoeba in recreational water bodies of Ilam province is of concern for high risk people. Increased public awareness and posting of warning sign or replacement of the recreational water sources at regular time should implicated.

Keywords: free-living amoeba, Vahlkampfiid, Acanthamoeba, water, Ilam

# PREVALENCE OF INTESTINAL PARASITIC IN-FECTION AMONG STUDENTS OF JAHROM UNI-VERSITY OF MEDICAL SCIENCES

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Considering that intestinal parasites play an important role in personal and social health care, finding infection sources in the various strata and their treatment is very important to prevent the spread of the infection. In the present study, the students of Jahrom University of Medical Sciences were examined for the intestinal parasites. The study was performed on 431 students selected randomly. Stool samples were collected from each student three times alternately and every time the samples were examined microscopically using both direct and formalin- ether methods.125 students (29%) were infected with intestinal pathogenic and non-pathogenic parasites. Prevalence of different parasites was determined as follows: Giardia lamblia 7.2%, Entamoeba coli 15.1%, Blastocystis hominis 12.3%, Enterobius vermicularis 0.7%, mixed infection with Blastocystis hominis and Entamoeba coli 0.9%, mix infection with Entamoeba coli and Giardia lamblia 0.5%, and mix infection with Blastocystis hominis and Giardia lamblia 0.2%. Although in some students infection was originated from outside the university, the carriers present among students was very effective for increasing the prevalence of infection. Finding the infected students through periodic tests in addition to treatment of infected inviduals will be useful to control the spread of infection.

Keywords: prevalence, intestinal parasites, students, Jahrom





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ISOLATION AND GENOTYPING OF ACAN-THAMOEBA SPP. FROM WATER SOURCES IN MAZANDARAN PROVINCE, NORTHERN IRAN IN 2013

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Acanthamoeba, a free-living amoeba is widely distributed in environment, water sources, soil, dust and air. It can cause karatitis and fatal granulomatous amebic encephalitis (GAE) in immunocompromised hosts. In this study 43 Acanthamoeba isolated from water sources in Mazandaran province, Northern Iran (Sari city and suburbs). Isolates were identified based on cyst and trophozoite morphological characteristics and PCR method. Appeoximately 500 bp region of the 18S rRNA gene (ASA.S1) including diagnostic fragment 3 (DF3) was amplified using the genus specific primers JDP1 and JDP2. Twenty positive isolates sent for sequencing. Results showed that 78% (14 samples) of all positive samples belonged to Acanthamoeba T4 genotype and 17.6% (3 samples) to A. Palestinensis T2 genotype. Of total fourteen isolates of Acanthamoeba genotypeT4, four isolates were defined as A. Rhysodes, two isolates were A. Polyphaga and three were defined as A. Castellani .Also three isolates were identified as A. palestinensis T2 genotype which were isolated from rice field and fish breeding ponds.Genotype characterization revealed abundance of Acanthamoeba spp in Sari city. More attention should be paied for visiting population annually and the immunocmpromised people.

Keywords: Acanthamoeba, genotyping, water sources, Sari, Iran

# ISOLATION OF ACANTHAMOEBA SPP. FROM DIFFERENT WATER SOURCES IN ISFAHAN

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Acanthamoeba spices are free-living amebas found in a wide variety of natural habitats. The high percentage of Acanthamoeba in different environmental sources represents a sanitary risk for public health especially contact lens users and immunocompromised patients. Acanthamoeba can also cause granulomatous amoebic encephalitis, otitis, lung lesions, and skin infections in immune deficiency individuals. In the present study, the status of contamination of the water sources of Isfahan is analyzed through parasitological method. The present study utilized 91 samples consisting 59 samples of tap water and 32 samples of environmental water from 15 different zones of Isfahan in May and June 2014. After filtering, cultivation was done in non-nutrient agar medium and was incubated at 30°C. The samples were analyzed based on morphological criteria. Acanthamoeba spp. were found in 71.9% of environmental water and 27.12% of tap water. Generally, Acanthamoeba spp. were found in 42.95% of samples. The results of the present study showed that the water contamination with Acanthamoeba spp. in different regions of Isfahan is potential infection source for high risk people. It could be suggested that public education and precaution are quiet necessary.

Keywords: Acanthamoeba, water, Isfahan





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MOLECULAR IDENTIFICATION OF ACAN-THAMOEBA SPECIES IN WATER PONDS OF PARKS AND SQUARES IN MASHHAD DURING 2012

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Free-living amoeba belonging to the genus Acanthamoeba is the causative agents of Granulomatous Amoebic Encephalitis and Amoebic Keratitis. Acanthamoeba spp. also have been associated with cutaneous lesions and sinusitis in AIDS patients and other immunocompromised individuals. They are distributed worldwide and have been isolated from soil, dust, air and stagnant water. Due to paucity of information about Acanthamoeba water contamination in Mashhad, we decided to investigate this issue in Mashhad. During December 2012 to June 2013, fifty samples were randomly collected from squares and pounds of parks of 13 regions of Mashhad, Iran and transferred to the laboratory of parasitology in Guilan University of Medical Science, Iran. Water samples were filtered and cultured on Non-Nutrient Agar (NNA) plates. Genomic DNA was extracted from positive samples in laboratory of parasitology of Ghaem hospital and PCR was performed to amplify the SSU-rRNA gene. Out of the 50 samples of the culture method in 31 cases (62%) of the freeliving amoeba was found in growth and reproduction. Using PCR, 11 samples (35.4%) were diagnosed with Acanthamoeba spp.which showed specific band of approximately 500 bp.In this study the prevalence of Acanthamoeba in the water ponds of parks and squares in Mashhad obtained 22%. It should be noted that regarding to relatively high prevalence of this contamination in our study and possible risks of these infectionsince the holy city of Mashhad annually receives millions of pilgrims- it is necessary that general practioners and public health organizations provide sufficient information for preventing of contamination. They should be aware of the possible role of water in the transmission of Acanthamoeba spp diseases.

Keywords: Acanthamoeba, Mashhad, PCR

PREVALENCE OF TOXOCARA EGGS IN SOIL SAMPLES OF WEST AZERBAIJAN PUBLIC PARKS

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Toxocariasis is a zoonotic disease caused by the larval stage of Toxocara canis and Toxocara cati which are the common roundworms living in the intestines of almost all new-born puppies, kittens and in some adult dogs and cats. Children are most commonly infected when they meet contaminated soil. Human Toxocariasis occurs after ingestion of infective eggs of Toxocara spp. and the subsequent migration of larvae, particularly to liver, lungs, muscles and brain, which causes Visceral Larval Migrans (VLM) and Ocular Larval Migrans (OLM). The aim of the present study was to assess the presence of Toxocara eggs in the soil of public parks West Azerbaijan. A total of 100 samples of soil were collected and examined from different environments of public health importance of West Azerbaijan including public parks and playgrounds. Fifty samples were from Urmia (5 different parks) and 50 samples from different parts of Azarbayjan were examined by modified flotation technique with sodium nitrate. About 27 samples out of 100 (27%) were found to be contaminated with the Toxocara eggs. 15 and 12 samples out of 50 samples from Urmia and Mahabad cities were positive, respectively. Nine parks out of 10 (90%) were contaminated with Toxocara eggs. Because of the relatively high contamination of parks of West Azerbaijan with Toxocara eggs and the potential risk of the infection to humans, preventive measures and further studies should be implemented inn this area. In addition, the local population should regularly be informed about the potential of acquiring zoonotic infections, advers affects of toxocariasis on their children and methods of prevention and control of the disease.

Keywords: Toxocara, soil contamination, West Azarbayjan





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# HUMAN TOXOCARIASIS: A SURVEY ON THE PA-TIENTS REFERRED TO HEMATOLOGY DE-PARTMENT OF LABORATORIES WITH HY-PEREOSINOPHILIA IN ARAK, IRAN

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Toxocariasis is an important zoonotic infection worldwide. The definitive hosts are dogs, cats and humans become infected when embryonated eggs are accidentally ingested. Larvae hatches in the small intestine, penetrate the intestinal wall and migrate, via the bloodstream, to the major organs and causes inflammatory responses and eosinophilia in blood. Four clinical syndromes are recognised: visceral larva migrans (VLM), ocular larva migrans (OLM), nervous larva migrans (NLM) and the other - mainly in adults- common toxocariasis (CT). Toxocariasis is one of the most important agents in eosinophilia. Due to the dramatic increase in the population of dogs in human dwellings in our country and since pet dog is kept by some families, this study aimed to investigate the prevalence of toxocariasis in hypereosinophilic patients and comparison was performed in people with normal eosinophily rate. This case-control study was performed on 100 hypereosinophilic patients (cases) and 100 people with normal eosinophily (controls) referred to hematology department of laboratories of Arak University of Medical Sciences, in one year. Epidemiological data were collected by questionnaire and diagnosis was performed using excreted-secreted antigens of Toxocara (TES Ag - ELISA). Positive toxocariasis in hypereosinophilic patients was 16% and in patients with normal level of eosinophil was zero (p=0/001). Toxocariasis in hypereosinophilic individuals had no correlation with gender, age, place of residence, occupation and education level of the patients (p > 0.05). Toxocariasis was significantly correlated with different history of contact with dogs and eating unwashed and non-disinfected fruits and vegetables.

Keywords: Toxocariasis, hypereosinophilia, laboratory diagnosis, Arak SOIL CONTAMINATION WITH TOXOCARA EGGS IN THE PUBLIC PARKS OF ISFAHAN, CENTRAL IRAN

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Human toxocariasis is a disease caused by the larval stage of Toxocara canis and T. cati. Humans are infected by ingesting the Toxocara eggs mainly through contact with contaminated soil. The aim of the present study was to evaluate the contamination of the public parks of Isfahan city with Toxocara ova. A total of 140 soil samples from 28 public parks of Isfahan city, were collected during summer 2014. Soil contamination with Toxocara eggs were investigated for the presence of Toxocara eggs by flotation method using sucrose solution. The prepared wet mount slides were examined by light microscope. Toxocara eggs were found in 21 (75%) out of 28 studied public parks. Toxocara spp. eggs were observed in 40 (28.6%) of 140 collected samples.Soil contamination rate with Toxocara eggs in Isfahan is fairly high. Isfahan is a touristic city with lots of parks and gardens in which the stray dogs and cats roam around and contaminate the soil, so preventive measures especially for children should be implemented in study area.

Keywords: Toxocara, soil, public parks, Isfahan, Iran





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SEROEPIDEMIOLOGICAL STUDY OF TOXOCA-RIASIS AMONG SCHOOLCHILDREN IN BU-SHEHR CITY, IRAN

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Introduction& Objectives: Human toxocariasis is a parasitic zoonosis caused by Toxocara canis and Toxocara cati. Infections in human hosts can occur upon accidental ingestion of embryonated eggs. Clinical manifestations depend on the organs affected by larva migrans. Despite the high prevalence of this parasitic infection, published information on the distribution of human toxocariasis is not enough. No information was available about prevalence of toxocariasis in Bushehr city, especially in children, who are the population at risk. ELISA is a reliable method to survey the seroepidemiology of the disease. This cross-sectional study conducted on 400 primary school students in Bushehr city. Blood sample was taken from each student and serum samples were stored at - 70°C after centrifugation. Anti-Toxocara IgG antibodies were measured by using a commercial ELISA kit. Samples were considered positive if the absorbance value was higher than 10% over the cut-off. The association between selected variables and seropositivity was analyzed by Chi square test. P< 0.05 was considered significant. A total of 400 school children in Bushehr were screened and 48 samples (12 %) were positive for IgG antibody against Toxocara. 19 (39.6%) out of 48 positive samples were male and 29 (60.4%) were female. From 48 students with positive test, 21 (44.8%), 9 (18.8%), 19 (39.6%), 6 (13%), 3 (6.2%), 1 (2.1%), 10 (20.8%), 12 (26.1%) and 5 (10.6%) cases complained of abdominal pain, anorexia, chronic cough, body pain, itching, wheezing, weakness, headache and nausea respectively and 13 (27.7%), 1 (2.1%), 8 (17%), 34 (72.3%), 16 (34%) cases stated that they have a habit of play ground, pica, onychophagia, thumb sucking, washing hand with soap and washing vegetables with detergent respectively. The results showed no significant association between different clinical manifestation, risk factors and Toxocara seropositivity. Conclusions: Total seroprevalence rate of toxocariasis was 12 percent. The results of our study showed no significant association between different clinical manifestation, behavioral and risk factor and Toxocara seropositivity. Also religious behaviors in Moslems, especially in Bushehr, that forbid them of close contact with dogs may be the second reason for low frequency of seroprevalence of toxocariasis in schoolchildren of Bushehr city.

Keywords: seroepidemiology, toxocariasis, IgG antibody, school children

## CASE REPORT OF NEW RICTULARIA SPECISE FROM STRAY DOG IN IRAN

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*Rectularia* (Spirorid, Rectularidae) is a common intestinal parasite of rodents and carnivores in many parts of the world. The length of parasite is about 4 to 9 mm and has 2 row of combs (about 90 to100 pairs). *Rictularia* is uncommon parasite of dogs in Iran. Three species of *Rictularia* have already been determined in Iran, including *Rictularia ratti*, obtained from rat in south, *Rictularia affinis*, from stray dogs in central and *R. cahirensis* from jackals in north of Iran.

During the study of intestinal parasites of stray dogs in Lorestan province, west of Iran, a nematode belongs to the Genus *Rictularia* was obtained. It is somewhat similar to species of *affinis, cahirensis* and *splindida* but different in some main characters such as the number of combs, spicules and fans and arrangement of caudal papillae. For these reasons, it seems to be new species of *Rictularia*.

Keywords: Reticularia, stray dogs, Iran





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## EFFECTIVE, APPROPRIATE AND SIMPLE CUL-TURE, EGG HATCHING AND CRYOPRESERVING OF OF THE NEMATODE CHEILOSPIRURA HAMULOSA

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Successful invasion of nematode parasites is associated with several factors including egg hatching at the right time in their hosts. To determine a simple and appropriate medium for culture and egg hatching of the highly pathogenic species of the Acuariidae family, Cheilospirura hamulosa were cultured in three different media. In addition, the viability of C. hamulosa eggs was determined after storage in frozen infected gizzards. Eggs removed from the uteri of the female worms in infected gizzards were pooled and washed in distilled water and screened under a stereo dissecting microscope. Eggs were counted and cultured in three different media, nutrient agar, normal saline 0.9% and Baermann, at room temperature. Additionally, 10 infected gizzards were kept at -20°C for 2 and 8 m.After 4-5 days, there had been no growth in the nutrient agar medium, whereas 11% of the cultured eggs in the Baermann medium contained larvae 2-3 days after culturing. In normal saline 0.9% medium the two polar knobs appeared on the two poles of the eggs at 2 days post cultivation, and 74% of the eggs contained a larva on the third day. Mature larvae gradually exited from the eggs. Eggs collected from female worms in gizzards frozen at -20°C were cultured in the same three culture media at room temperature. Larvae were visible in the eggs after 2-3 d in the Baermann medium and normal saline 0.9% media and hatched thereafter. The 0.9% normal saline medium is recommended for egg hatching and cultivation of C. hamulosa due to the simplicity, efficacy and cost effectiveness. Moreover, freezing of the infected gizzards at  $-20^{\circ}$ C is proposed for long-term storage of the eggs.

Keywords: medium culture, nematodes, Cheilospirura hamulosa

# PREVALENCE OF GONGYLONEMA PULCHRUM IN CATTLE SLAUGHTERED IN MASHHAD AB-ATTOIR

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Gongylonema pulchrum is a nematode from spirurida order. The life cycle of this nematode is indirect and the final host is infected by ingestion of infected poultry or insects. It is usually found in the upper digestive tract. In recent studies, there have been more than 50 human cases reported all over the world as well as Iran. Symptoms include local irritation, pharyngitis, stomatitis, and bloody oozing patches in the mouth. Materials & Metods: The present study was performed on 300 cattles slaughtered in Mashhad abattoir. Of 300 studied cattle 20 (6.6%) were infected with Gongylonema pulchrum and 7 male and 45 female nematodes were collected from oesophagus of infected cattle. In one infected cow 14 adult Gongylonema were obtained. In severe infections swelling of the mouth, deformation of esophagus was found. Gongylonema pulchrum makes neoplastic changes of the esophagus.Since this parasite is zoonoses having the potential for infecting humans, further investigation of the parasite is recommended.

Keywords: Gongylonema pulchrum, cattle, Mashhad





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## THE FIRST CASE OF CAPILLARIA HEPATICA IN-FECTION IN RATTUS RATTUS FROM SANANDAJ, KURDISTAN, IRAN

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Capillaria hepatica is parasitic nematodes of the order Trichinllidea that could be accidentally transmitted to humans and cause zoonosis capillariasis. This parasite lives in the liver parenchyma and reports a rare but severe and fatal infection in humans. In the case of human infection, symptoms such as splenomegaly, acute liver inflammation, peritonitis, ascites and eosinophilia may occur. Active life cycle of the parasite in different geographical locations can be a potential risk for the community's health. This report is the first case of Capillaria hepatica infection in Rattus Rattus in Sanandaj-Kurdistan, One Rattus rattus was taken from the city of Sanandaj and transferred to the Parasitology laboratory. After dissection we suspected to abnormal liver appearance with rough unusual colored spots on the surface. Following further scrutiny with a microscope, eggs and adult worms were found in the liver parenchyma. The worms were carefully isolated from the liver parenchyma obtained material transferred to falcon tube. Then, the tube washed 2 times with distilled water and centrifuged in 1000 rpm and the eggs were isolated. Result: Brown oval shaped egg with the size of approximately 55 microns and shallow polar knobs at both ends with double layer shell and minipores visible in the outer shell were seen. The adult worms were approximately 35 mm in length, which seems to be different from the size of the parasites reported from other areas. The results of the present study verify the presence of Capillaria transmission cycle in Sanandaj area of Kurdistan and the necessities for implementation of appropriate measures to control the rodent population.

Keywords: parasites, Capillaria hepatica, zoonosis, rodents, Sanandaj

## PREVALENCE OF MACRACANTHORHYNCHUS HIRUDINACEUS IN WILD BOARS IN BUSHEHR PROVINCE, SOUTHWEST OF IRAN IN 2014

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Wild boars (Sus Scrofa) are in close contact with human populations, especially in rural communities and may potentially act as sources of zoonotic diseases. Macracanthorhynchus hirudinaceus is a zoonotic acanthocephalan helminth which lives in small intestine of boars and may transmit to human via accidently ingesting of its insect intermediate host. The current study aimed to evaluate the prevalence of M. hirudinaceus in wild boars in Deylam district in Bushehr province, South of Iran. This cross-sectional study conducted on 25 crossbred hunted boars form May to October 2014 in Deylam district. After hunting, internal peritoneal contents were removed and colon and small intestine were cut from Ileocecal valve. The small intestine was opened with scissors and acanthocephalan helminthes were removed from the intestine. Collected helminthes were put in running water for six hours followed by transferring into hot alcohol, formalin, acid acetic (AFA) till the helminthes were killed and fixed. Finally, samples were preserved in alcohol-glycerin solution, and stained by Carmine Alum. From 25 hunted boars, 11 (44%) were male and 14 (56%) were female. Mean age of the hunted animals was 3.4 years (maximum age was 9 and the minimum was 1year), and mean weight of the animals was 131 Kg (45-275 kg). Prevalence of M. hirudinaceus was found to be 52% in hunted boars. Male constituted 54.5% of the infected animals while female constituted 50% of the infected boars. Findings of this study showed a high prevalence of M. hirudinaceus infection in wild boars in Bushehr province. This may impose potential risks of infection on farmers and residents of rural areas.

Keywords: Macracanthorhynchus hirudinaceus, wild boar, Bushehr





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GEOGRAPHICAL DISTRIBUTION OF THE HEL-MINTHIC DISEASES COMMON IN THE MA-ZANDARAN PROVINCE, NORTHERN IRAN FROM 1981 UNTIL 2013

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Infectious diseases, particularly parasitic helminthic diseases, are importand in terms of health care due to the appropriate climatic and geographical conditions in the province. Therefore, GIS can be used to define the geographical distribution and the status of these diseases on the map. The present study aimed to investigate the geographical distribution of parasitic worm infections commonly occured in the province from 1991 to 2013. This descriptive study performed by the review of papers published in journals, papers presented at congresses, summary of the dissertation and the use of the Internet network to collect information on search engines. Data collected for each type of parasitic helminthic infections, place of publication, year of the report, the percentage of infection (prevalence), article title and author, were classified. The table or tables associated with each of parasites were prepared and geographical maps using GIS software ArcGIS terms laid out in the city. The results of data classification using the resources of the study, indicated the most common parasites. Province included 1 - Ascaris lumbricoides, 2 – Trichuris trichiura, 3 – Enterobius vermicularis4 – hook worm disease, 5 - Strongyloides stercoralis 6 - Trichuristrongylous, 7 -Dicrocoelium, 8 - Fasciola spp. 9 - Taenia saginata, 10- Hymenolepis nana, and 11 - the hydatid cyst disease which are divided in three groups : nematodes, cestodes and trematodes. The results of this study indicate the difference in the prevalence of parasitic worm infections, according to the study groups and different regions in the province. By calculating the average amount of each disease, worm infestation on the map, changes of the trend of the diseases and not reported illnesses, areas with low moderate and high prevalence have been identified.Classification and preparation of plans for distribution of these infections can not only help to better understand and interpret the situation but also presents information on distribution and trend of the disease in the last twenty years, causes of outbreaks and how they spread.

Keywords: geographical distribution, helminthic diseases, Mazandaran, Iran

## SEROPREVALENCE OF TOXOCARA INFECTION AMONG ADULT INDIVIDUALS WITH EOSINO-PHILIA IN BABOL DURING 2013

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Toxocariasis is a worldwide zoonotic disease caused by two species of the Genus Toxocara, T. canis and T. cati. Hypereosinophilia is a common picture of the disease. The aim of the present study was to determine the seroprevalence of Toxocara among healthy adult individuals with eosinophilia over 10%, referred to health center of Babol and also to find out the risk factors of the infection. In this cross-sectional descriptive study, 322 healthy adult subjects with over 10% eosinophilia, aged above 15 years old, were selected. Overall, 9865 serum samples were collected and evaluated with indirect ELISA technique for presence of specific IgG antibody against Toxocara canis. Statistical analysis of the data was performed using Chi square test with SPSS 16. The overall seroprevalence of Toxocara infection among adult individuals with eosinophilia was 23.5% (76/322). Among them, 45 (59.2 %) were male and the rest 31(40.8 %) were female. Our result showed significant differences between place of residence (higher rates in rural areas) (p=0.001) and job (higher rates in farmers) (p=0.005).Our study showed that farmers and people living in rural areas are at higher risk of toxocariasis. Moreover, the present study offers increasing popular awareness about this disease and also more efficient control of stray dogs.

Keywords: toxocariasis, seroprevalence, eosinophilia, ELI-SA, Babol





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#### INTRA- ABDOMINAL TAENIASIS: A VERY RARE CASE REPORT

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Taenia saginata is a zoonotic parasite causes gastric complication in humans (Taeniasis). Usually, the disease is confined to the terminal parts of small intestine but ectopic cases have also been reported. The present study describes a very rare case of ectopic Taeniasis. A 42-years old man known as a dermatomyositis case living in Babol was admitted to Rouhani hospital, Babol, northern Iran. He had a history of hospitalization due to flare up of dermatomyositis five months before admission and treated with cyclosporine and prednisolone. Patient medications at admission were prednisolone, azathioprine, methotrexate, and rituximab. The patient complained of severe epigastric pain which started one week ago and become severe from 24 hours before admission. He also complained of loss of appetite. In physical examination, generalized tenderness in the abdomen, especially in the right lower quadrant without guarding or organomegaly were observed. He had no fever. His hemoglobin level was 9.1 gm/dl and leukocytosis was not seen. Blood and stool cultures were negative. The amount of CRP and ESR were 135mm/h and 86, respectively. Level of amylase and lipase were normal. Abnormality was not observed by colonoscopy and sonography. Thickening of cecum and terminal ileum wall without mass were reported by CT scan. Due to the lack of recovery, diagnostic laparoscopy was performed, which indicates inflammation and fibrin around the cecum. McBurney incision was carried out in order to perform an appendectomy. Two worms were found in the abdominal area before cutting the appendicitis. They were approximately 15 mm long and 4 mm wide. The worms were examined by microscopy method and Taenia saginata was diagnosed. The patient was treated with niclosamide and symptoms completely disapeared after treatment. The case was followed up for six months. Stool examination was performed repeatedly during the six months and no parasitic infection was reported. Also, similar symptoms and signs were not observed.

Keywords: Taenia saginata, Taeniasis, intra-abdominal, Iran

IMMUNOGENIC CROSS-REACTION AMONG EX-CERETORY-SECRETORY PROTEINS OF TOXO-CARA SPECIES

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Toxocara canis causes human visceral larva migrans syndrome. T. canis antigens make challenges with host immune system. The aim of the present study was determination of immunogenic bands and comparison the cross reactions of the antigens. T. canis, T. cati and Toxascaris leonina was isolated from dogs and immediately after biopsy, they were washed with normal saline 37° C and cultured in RPMI containing antibiotics. Exceretory- secretory antigens of the worms were collected during 72h. Protein measurement was performed by Bradford reagent and evaluated by SDS-PAGE. E-S antigens and somatic whole antigens with complete freund adjuvant and with incomplete freund adjuvant (two times) was injected to rabbis and blood samples were collected 4 weeks later. Western blot was performed with rabbis' sera in 1:100 and 1:200 dilutions. The results of Western Blot provided in five bands which 28 KDa and 57KDa bands appeared with higher intensity and concentration. T. canis and T. cati reacted with rabbit anti E-S Antigens sera as well rabbit anti somatic antigens sera of T. canis, but Toxascaris leonina showed just a weak band in 57KDa.E-S antigens of T. cati reacted with rabbit anti T. canis sera. These are not species-specific antigens. In the Ascariidae family these antigens are not genus specific. The results of other studies have shown E-S antigens of Toxascaris leonina and Ascaris lumbricoides reacted with rabbit anti T. canis sera, these parasites reacted with 57KDa protein. The results of the present study could be used for the screening of the specific antigens.

Keywords: *Toxocara canis, Toxocara cati,* exceretory- secretory antigens, cross reactions, western blot.





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#### PREVALENCE OF CYSTICERCUS FASCIOLARIS AMONG WILD RODENTS IN BOYERAHMAD TOWNSHIP IN 2014

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Rodents can serve as reservoirs for different diseases, especially for those parasitic infections which infect humans. Helminthic infections are considered as a potential risk for humans. Cysticercus fasciolaris is the larval stage of the cestode Taenia Taeniaeformis, which its adult worm exists mainly in cats. This larval stage considered as intermediate hosts for a variety of small rodents and sometimes for humans and birds. This study aimed to assess the prevalence of Cysticercus fasciolaris among rodents hunted in Bouyerahmad province in 2014. Material & Method: Fifty two wild rodents were hunted by Sherman live traps, containing baits from July 2014 to December 2014 in different parts of Boyerahmad Township. Rodents were anesthetized by ether and killed in accordance with the ethical principles of working with laboratory animals. Animal necropsy was performed and components within the peritoneal cavity were removed and different organ including liver, lung and stomach were grossly examined for presence of parasites. Finally, according to the parasite's diagnostic keys, the larvae isolated from various organs were examined and identified. The average weight of the rodents was 125 gr. In this study the infection with Cysticercus Fasciolaris was found in 3 cases (5.8%). Findings of this study show a relatively high prevalence of infection among wild rodents in Bouyerahmad Township which might be a potential risk for habitants of these areas.

Keywords: Cysticercus fasciolaris, wild rodents, Bouyerahmad Township

## EVALUATION OF COUNTERIMMUNOELEC-TROPHORESIS FOR DETECTION OF LINGUAT-ULA SERRATA INFECTION IN SHEEP

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Linguatula serrata is a cosmopolitan and zoonotic parasite. Adults occur in the nasal and respiratory passages of dog and other canids. Herbivores (sheep, goats, etc.) are intermediate hosts of the parasite. Human may become infected by eating the eggs or nymphs of the parasite. Larval stages infections of Linguatula serrata in herbivores are clinically symptomless and until now there were no any reports of serological methods in diagnosis of the parasite infection in intermediate hosts. For the first time, in present study counterimmunoelectrophoresis (CIEP ) was evaluated for diagnosis of Linguatula serrata infection in sheep using nymphal somatic (S) and excretory/secretory (ES) antigens (cultured in RPMI), 3 different buffers (pH,7.7) and 40 positive sera (from the natural infected animal), 30 negative sera (from indoor lambs). Some quantitative indicators of the test (CIEP) with both Veronal and Tris-tricine buffers were determined by using ES antigens of the cultured nymphs of the parasite in RPMI. The CIEP test using ES antigens showed 100% sensitivity and specificity with both buffers. The results of CIEP usings S antigens showed 100% specificity with both buffers and sensitivity 100% (with Veronal buf.) and 95% (with Tris-tricine buf.). Sensitivity of the antigens were 100% and 95% with the buffers respectively. Sensitivity and specificity of the test with barbital buffer were 92.5% and 90% for ES antigens, 97.5% and 86.6% for S antigen. The results of the present study showed that CIEP test can be used to detect Linguatula serrata infection in sheep by using ES and S antigens of nymphal stages of the parasite with Veronal and Tris-tricine buffers. The test can be developed for other animal hosts and for human.

Keywords: Linguatula serrata, counterimmunoelectrophoresis, diagnosis, Iran





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A STUDY ON EPIDEMIOLOGICAL CHARACTER-ISTICS OF SCORPION ENVENOMATION IN KHORAM-SHAHR, IRAN: AN ANALYSIS OF 903 CASES

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Scorpions are most common in warm climates, especially in areas considered to be semi-arid. These creatures are common in rural and urban environments of Iran. Scorpion sting is one of the important health hazards in the province of Khuzestan. The aim of this study was to describe the epidemiology among humans stung by scorpion in the County of Khorram-shahr, southwestern Iran. This descriptive crosssectional research was carried out during 2007-2009. Epidemiological data were recorded in a questionnaire and analyzed by SPSS software. A total of 903 cases of scorpion envenomation were reported during three years interval. The mean incidence of scorpion stings was 2.4/1000/3 years. Almost 59.5% of cases were men. Although scorpion sting occurred in all months of the year, most of stings appeared in summer and the least in winter. Most of stings (34.2%) were seen in age group of 15-24 years old. Among these patients, 69.3% were from urban areas and 30.7% from rural regions. The highest cases of stings (45.2%) occurred in the hand and the least (13%) in the head and body of the victims.Scorpionism in Khoram-Shahr County is a public health problem, and needs to be monitored very carefully.

Keywords: epidemiology, scorpion sting, Iran.

PREVALENCE OF HEAD LICE AND ITS RELAT-ED FACTORS IN THE PRIMARY SCHOOL STU-DENTS IN GLOGAH COUNTY, MAZANDARAN PROVINCE, NORTHERN IRAN

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Head lice infestation (pediculosis capitis) is one of the most important health problems of students. Close Physical relationships between students in schools provide suitable conditions for the transmission of this parasite. The existence of these parasites in students in addition to creating health problems, results in psychological influences and social pressures. This study was conducted in order to determine prevalence of pediculosis capitis and some effective factors on it among pupils in primary schools of Glogah County. This descriptiveanalytic study was conducted in eight primary schools in the urban areas and eight primary schools in the rural areas during 2009-2010. Totally, 300 students of the first - fifth grades were selected by stratified cluster sampling and examined by experienced trained personnel. Data were recorded in a questionnaire and analyzed by SPSS software. Finally, the results were compared with chi square test. From all of the 300 pupils, 17 (5.7%) were infested with pediculosis capitis. From this infested pupils, 8 patients (47%) were female and 9 (53%) were male. The majority of infested pupils (64.7%) was in age group between 9-11 years old. The highest prevalence of infestation was observed in students whose mothers and fathers were farmers (14.3% and 23.5%, respectively). A significant difference was observed between the amounts of head lice infestation in students with the health care status of children, the use of common equipment, the status of life of children with both parents, knowledge of the lice, bathing week frequencies, attendance of health instructors in school, approximated space used by students in the classroom and frequencies of combing the hair daily.Results showed that prevalence of pediculosis among Glogah county primary school students was less than average percentages observed in other students inside and outside Iran.

**Keywords:** pediculosis, head lice, pupils, epidemiology, related factors, primary school, Iran.





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# ECTOPARASITOSIS IN PATIENTS REFERRED TO PARASITOLOGY LABORATORY OF IMAM REZA HOSPITAL OF MASHHAD DURING 1995-2011

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Ectoparasites that cause conditions such as pediculosis, scabies and myiasis, are a group of parasites that live on the surface of their hosts. These diseases are common in Iran. The purpose of this study was to evaluate the epidemiology of these diseases in suspected patients in Mashhad. This crosssectional study was performed on all patients suspected to ectoparasitosis referred to Parasitology Mycology laboratory of Imam Reza hospital of Mashhad during 15 years from 1995-2011. Data were collected based on the questionnaires and then analyzed statistically. In this study from 1851 suspected patients to ectoparasites, 375 had scabies, 99 had pediculosis and 61 had myiasis. The mean age of patients was 26.18±17.68. The most common age of scabies was 10-19 (27.7%) and pediculosis 0-9 (9.6%) (P = 0.00). The highest incidence of pediculosis was in women (7.3%) and scabies in men (28.1%) (P = 0.00). In terms of occupation, pediculosis was most common in students (9.9%) and scabies in workers (32%) (P =0.00). Scabies was more common in patients from North Khorasan (28.6%) and pediculosis in South Khorasan (10%) (P =0.08). 62.3% of patients with myiasis were males and 37.7% were female. Approximately 50% of patients were under the age of puberty. Aside from children and students who constitute the greatest number of afflicted case, next highest rate of occupational affliction belongs to the shepherds. 100% of myiasis infections occurred in the first 6 months of year. More than 55% of patients were infected with primary myiasis. The most common etiologic agent was Oestrus ovis larva which was isolated from 37.7% of the cases.Based on the results, contamination with ectoparasite depends on age, gender, location and occupation and myiasis is more common in the first 6 months of year.

Keywords: ectoparasitosis, pediculosis, myiasis, scabies, Iran

# EVALUATION OF TICK BITES ACCORDING TO ANATOMICAL REGIONS ON HUMANS IN THE LIGHT OF THE STUDIES IN TURKEY

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Ticks are obligate hematophagous arthropods that parasitize every kind of vertebrates in almost every region of the world and can cause anemia, toxication, paralysis, irritation, allergy and also secondary infection because of skin lesion. Ticks are biological and mechanical vectors of varies viral, bacterial, rickettsial, spirochethal, protozoan and helmintic diseases. The aim of this study was to evaluate tick bite cases according to anatomical regions on humans in the light of the studies in Turkey. Tick bite cases in Turkey were screened in literature. According to literature it was seen that anatomical regions were explained in 4413 cases for tick bites. Results were evaluated for anatomical regions as percentage. Most of the tick bites were seen on pars membrum superioris and inferioris 1758 (39.83%), cranium- cervical region 973 (22.04%), thorax and abdomen 924 (20.93%), regio genitalia / inguinalis 447 (10.12%) and rarely on regio dorsalis / omos 88 (1.99%), regio axillaris 80 (1.81%), auricula 55 (1.24%), regio pedis 37 (0.83%), gluteal region 36 (0.81%), palpebra 7 (0.15%), meatus acusticus externus 7 (0.15%) and conjunctiva 1 (0.02%).Tick bite cases in Turkey were completely evaluated firstly according to anatomical regions and it is suggested that all body regions should be examined among tick bite.

Keywords: tick, anatomical region, Turkey





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#### A SURVEY ON HARD TICKS FAUNA IN KALAT NADERI OF KHORASAN RAZAVI PROVINCE IN SPRING 2014

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Introduction & Objectives; Ticks are vectors for transmission of pathogens to the humans and animals. Ticks are important vectors of blood-transmitted diseases, including Babesioses and Theileriosis in sheep and cattle, viral diseases such as Crimean-Congo hemorrhagic fever and spirochetosis in livestock. The aim of the present study was to investigate the ticks fauna on animals in Kalat Naderi city in spring 2014. In the March and April 2014, according to the topographic of study region, 12 villages in Kalat Naderi were sampled.Four samples were randomly collected from the central part, four from Zavin District and four from Lain district. The Genus and species of the collected ticks were identified using diagnostic keys. A total of 136 ticks collected from these areas, incloding 85 female ticks (62.5%) and 51 male (37.5%). The male ticks were classifid into tow genera and four species including: Hyalomma marginatum (45.1%), Hyalomma anatolicum anatolicum (31.3%), Dermacentor raskemensis (15.7%), Dermacentor marginatus (7.9%). Hyalomma Genus was more common (with a frequency of 76.4%). Climatic conditions play an important role in growth of some species of hard ticks, Hyalomma and Dermasentor which are potential vectors of Theileriosis and Babesiosi. Due to the occurrence of babesiosis and theileriosis in the study area, and Crimean Congo haemorrhagic fever (CCHF) in Khorasan Rasavi province, regular surveillance of ticks seems necessary for control and prevention of abovementioned diseases.

Keywords: hard ticks, fauna, Kalat Naderi, Khorasan Razavi.

ECOLOGY AND BIOLOGY OF HOUSE DUST MITES, THE CAUSAL AGENTS OF ALLERGIES: A REVIEW ARTICLE

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House dust mites are in Class Arachnida, Subclass Acari, Order Astigmata and Family Pyroglyphididae. The most important genus in this family is Dermatophagoides. About 20 species of mites are found in house dust. The Dermatophagoides species are very similar but have differences in some physical characteristics. The most important house dust mites are Dermatophagoides pteronyssinus and D. farinae. The life cycle of these two mite species include egg, active larva, resting larva (pharate tritonymph), active tritonymph, resting tritonymph (pharate adult) and active adult. D pteronyssinus females weigh approximately 0.2 - 5.8 mg (fresh weight) whilst males are approximately, 0.2 - 3.5 mg. Dermatophagoides mites are very small (0.3 mm) and live among carpets, bedclothes, mattresses, and general house dust. Optimal conditions for development of eggs and emergence of adult mites are 23°C and 75% relative humidity. The duration of the life cycle from egg to adult for male is 30 days and for female approximately 70 days, but these periods depend on the temperature and humidity of the environment. D. farinae lays eggs over a 30day period, producing about an egg a day, while D. pteronyssinus lays about 80 - 120 eggs over a 45-day period. House dust mites feed on human skin scales, pollen, fungi, bacteria and animal dander. Densities above 100 mites per gram are considered a risk factor for sensitization to allergies such as bronchial asthma, rhinitis, conjunctivitis and sometimes atopic eczema, while 500 mites per gram is a major risk factor in the development of acute asthma in those allergic to housedust mites.

Keywords: house-dust mites, *Dermatophagoides pteronyssinus*, *D. farina*, allergy, asthma





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## A RETROSPECTIVE STUDY OF DEMODICIDIOSIS IN PATIENTS REFERRED TO IMAM REZA HOS-PITAL OF MASHHAD DURING 12 YEARS

## <u>Fariba Berenji</u><sup>1</sup>, Abdolmajid Fata<sup>2</sup>, Mahmoud Parian<sup>3</sup>, Maryam Nakhaei<sup>3</sup>, Narges Khazaee<sup>4</sup>

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Demodex folliculorum is an obligatory parasite in the hair follicles and in the pilosebaceous glands. Demodex mites are being implicated as the causative agents of some forms of rosacea, perioral dermatitis and blepharitis. The aim of this study was to document the descriptive epidemiological features of demodicidiosis in 1272 patients referred to Imam Reza hospital during a 12 years period. During 12 years (Mar 2000-Dec 2014), patients with skin lesions suspected of having Demodex folliculorum infestation, were examined in parasitology laboratory of Imam Reza hospital. All cases were referred by dermatologists and had clinically suspicious skin lesions. Samples were taken by scalpel from skin lesions and fresh smears with 10% KOH were prepared and examined directly under light microscope. Of 1272 patients suspected for demodicidiosis, 708 cases (55.7%) were positive, 448 cases (63.2%) were female and 260 (36.8%) male. The highest rate of infestation was observed among the age group 30-40 years and the lowest rate among 0-10 years. Most of the patients were housewives (53%).Demodicidiosis is one of the most prevalent skin diseases in Iran. In our study higher prevalence rate was seen in female and in age group 30-40. The authors believe that many adult acne and peri-oral dermatitis and belepharities conditions may be found to have an infestation of Demodex folliculorum and it should be considered as an important agent in these lesions.

Keywords: Demodex folliculorum, demodicidiosis

## REPORT OF ARGULUS INFESTATION OF GOLD-FISH IN ZABOL

# Maryam Ganjali\*1, Mahdie Mishmast<sup>2</sup>, Rreza Nabavi<sup>1</sup>

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The goldfish (Carassius auratus auratus) is a well-known freshwater fish of the family Cyprinidae with a worldwide distribution. Due to increasing interest of keeping aquarium fish has clarified the neccessity for identification of goldfish diseases. Importing the fish from other countries, carrying unidentified pathogens and parasites, may play a role as the source of infection for other local fish. Argulus is one of the most widespread crustacean ectoparasite of freshwater fish. The parasite feeding cause skin irritation, tissue damage, stress and secondary infections in fish. This report is introducing the ectoprasites collected from the tail and fin of goldfish studied at the department of parasitology, university of Zabol. Examinations under stereomicroscope using the available diagnostic keys the parasites were identified as Argulus. The parasite size was from 3-6 mm in length and 2-5 mm in width. A typical fish louse of the genus Argulus is very flat with an oval or rounded carapace, four pairs of legs, sucking mouthparts with a piercing stylet, and two suction cups to attach to its host.

Keywords: goldfish, Carassius auratus auratus, cyprinidae,





Proceedings of 2nd International and 9th National Congress of Parasitology and Parasitic Diseases of Iran (NICOPA 9)

Guilan, Iran; 20-22 May 2015 **REPORT OF SUBCUTANEOUS MYASIS DUE TO IDENTIFICATION OF HARD TICKS OF RABBITS** GASTEROPHILUS NASALIS IN DEER IN ZABOL COUNTY, IRAN Maryam Ganjali<sup>1</sup>, Mojtaba Keighobadi<sup>2</sup> Maryam Ganjali<sup>1</sup>, Mojtaba Keighobadi<sup>2</sup> \* 1-Assistant Professor, Department of Parasitology, Faculty of \* 1-Assistant Professor, Department of Parasitology, Faculty of Vet-Veterinary Medicine, University of Zabol, Iran. m.ganjali@uoz.ac.ir erinary Medicine, University of Zabol , Iran m.ganjali@uoz.ac.ir 2- Instructor, Institute of Specific Animals, University of Zabol, 2- Instructor, Institute of Specific Animals, University of Zabol, Iran Iran Myiasis is defined as the infestation of human and animal The ticks are obligate blood-feeding parasites which may cause anemia. Different species of ticks play an important tissues by fly larvae. Infection may occur through skin wounds or body cavities such as the mouth, nose, eyes and role in transmission of tick borne diseases resulting in animal and human health problems and death. Ticks infestations genital opening. Fly larvae infestation may cause lots of complications which affect animal and human health and even have been reported from domestic and wild animals. Rabbits mortality. In accidental myiasis, external parasites invade inalso are known as the host of different species of ticks. Ticks appropriate hosts or their eggs may accidentally be ingested. can transmit diseases such as tularemia and may cause mor-Gasterophilus is a genus of parasitic flies in the family Gasterotality in rabbits. In this survey sampling was conducted from philidae which cause obligatory myasis. Gasterophilus spp. usudifferent part of zabol. Ticks were collected from the head ally lay eggs on equines, they commonly known as the horse and neck of infested animals and stored in 70% ethanol, then bot fly and distributed worldwide. Myiasis similar to that of transported to the laboratory of Faculty of Veterinary Medi-Hypodrema may rarely appear in humans and other vertebrates cine of Zabol University. Following examinations under steby Gasterophilus larvae. In this case report, a swelling was obreomicroscope, ticks were identified using available keys. served on the leg of a deer at Sistan zoo in physical examina-Only one species of Rhipicephalus turanicus were identified and tion. At Faculty of Veterinary Medicine of Zabol University, the ratio of male ticks were higher than the females. fly larvae were removed by cutting and draining the lesion and identified as Gasterophilus nasalis using diagnostic keys. Keywords: hard ticks, Rhipicephalus turanicus, rabbit, Keywords: myiasis, Gasterophilus nasalis, deer





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PREVALENCE OF PEDICULOSIS IN ELEMEN-TARY SCHOOLS IN ALIABAD CITY, GOLESTAN PROVINCE, 93-92

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Head lice infection is seen at any age, sex and socioeconomic levels. Schools are one of the most common places around the world where head lice spreads. This study is done with the aim to identify children infected with head lice and its associated factors among school children of Aliabad Katol. Materials and Methods: This cross - sectional study with descriptive-analytical approach has been done on 252 cases which have been selected through multistage cluster sampling of elementary school students of Aliabad Katol in the academic year 92-93. Data were collected through examination on hair and questionnaire. Data analysis was done by using  $\chi^2$  and Fisher's exact tests and Kramer correlation coefficient in SPSS-19 software and P<0.05 was considered significant. 127 girls and 125 boys were examined. The infection rate was estimated to be 11.9% in primary schools of Aliabad Katol. The infection rate was 5.6% in boys and 18.1% in girls. Statistical tests of residence, maternal education, ethnicity and gender showed a significant effect on the incidence of pediculosis (P<0.05).Improving the health awareness of parents specially mothers and female students in rural areas is essential for prevention and reduction of the infection.

Keywords: prevalence, pediculosis, elementary schoolchildren

INVESTIGATION OF PROTEASE ACTIVITY IN THE EXCRETORY-ECRETORY RELEASE FROM THE NYMPHAL STAGE OF LINGUATULA SER-RATA

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Linguatula serrata is a zoonotic parasite known as the tongue worm. The adult form occurring in the upper respiratory passages, nasal airways and frontal sinuses of canines, felines and other carnivores. L. serrata eggs are infective for wide range of mammal intermediate hosts, particularly large grazing animals. Human infection occurs following ingesting raw or undercooked infected visceral organs of the intermediate hosts. Destruction of tissues in intermediate hosts happens due to the migration of nymphal stage. Proteases are interesting biomarkers for the detection of diseases and being presumably involved in processes such as penetration of host tissues, parasite nutrition, anti-coagulation and evasion of host immune responses as well. There is limited information available on the proteases activity in excretory- secretory products in L. serrata. The aim of this study was to evaluate the presence of proteases and determine the protease major activity in the ES release from nymphal stage of L. serrata.After collection of mesenteric lymph nodes of goats, each lymph node was cut longitudinally, put in the Petri dish with distilled water for 15 minutes, washed three times in saline phosphatebuffered (PH=7.2), and transferred to the Petri dish containing minimum essential culture medium. After incubation at 35°C with 5% Co2 for 24 h, nymphs of L. serrata were removed and medium was collected, and centrifuged (15 min, 5000g). Protease activity was determined, using 1% casein as substrates and the reaction was carried out at 37°C for 60 min. In order to determine major protease in the ES release from nymphal stage of L. serrata, protease specific inhibitors were used at various concentrations. Protease inhibitors used in this study were PMSF, EDTA, E64 and 1, and10 phenanthroline. Activity staining was performed according to the method of Garcia-Carreno, Dimes, and Haard. Crude proteases were separated on 10% polyacrylamide gel. The results of our studies showed that the EDTA and 1, 10-phenanthroline inhibitors reduced the proteolytic activity of E.S release from nymphal stage of L. serrata by a rate of 12.5% and 28%, respectively. In contrast, PMSF and E64 didn't have any effect on proteolytic activity, therefore, the main protease in ES release from nymphal stage of L. serrata was metalloprotease.

Keywords: Linguatula serrata, protease, metalloproteas, zoonotic





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Guilan, Iran; 20-22 May 2015 TICKS ATTACHING TO HUMANS IN BITLIS; A DETER

PRELIMINARY STUDY

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Ticks are obligate hematophagous arthropods that parasitize every class of vertebrates in almost every region of the world. Ticks can transmit approximately 200 diseases to humans and animals. This study aims to determine tick species detached from patients who complained tick bite in Emergency service in State Hospital in Bitlis province of Turkey. Ticks were removed from 91 patients who complained of tick bite during May and August 2009 in the Emergency Department of Bitlis State Hospital located in Eastern Anatolian region of Turkey. Ticks stored in tubes containing ethanol 70% and diagnosed under a stereo microscope according to the morphological features. Nine tick species were identified as follows: R. bursa (Canestrini & Fanzago, 1878) (18/91), D. marginatus (Sulzer, 1776) (15/91), H. anatolicum (Koch, 1844) (12/91), H. aegyptium (Linnaeus, 1758) (9/91), H. sulcata (Canestrini & Fanzago, 1878) (9/91), H. marginatum (Koch, 1844) (6/91), H. excavatum (Koch, 1844) (6/91), R. sanguineus group (6/91), Haemaphysalis spp. (3/91) and H. rufipes (Koch, 1844) (1/91). In addition to this two Rhipicephalus sp. nymhs and four Hyalomma sp. nymhs were identified. These results provide first data for tick species infesting humans in Bitlis province. Further studies are required to determine which tick species are infesting humans and domestic-wild animals in Turkey, and also throughout the world.

Keywords: tick, human, Bitlis, Turkey

DETERMINED TICK SPECIES ON CATTLE, SHEEP AND GOATS IN TURKEY: SEASONAL AC-TIVITY AND GEOGRAPHIC DISTRIBUTION

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Ticks have many direct and indirect harmful effects on human and animals. Over 200 diseases are transmitted by ticks all over the world. More than 30 tick species have been identified in Turkey. Tick studies conducted on cattle, sheep and goats in Turkey were screened in literature. According to 28 literatures related to subject; species, seasonal activity and geographic distribution of ticks were determined. According to the studies conducted related subject; Rhipicephalus (Boophilus) annulatus, R. (B.) kohlsi, R. bursa, R. sanguineus, R. turanicus, Dermacentor marginatus, D. niveus, Hyalomma anatolicum, H. excavatum, H. turanicum, H. marginatum, H. impeltatum, H. detritum, H. rufipes, H. aegyptium, Haemaphysalis concinna, H. inermis, H. parva, H. punctata, H. sulcata, Ixodes ricinus, I. hexagonus, , Ornithodoros lahorensis and Otobius megnini were identified on cattle, sheep and goats in Turkey. However, limited information was obtained about seasonal activity for ticks. It was concluded that further studies should be performed to determine tick species and seasonal activity on livestock and this can be helpful to develop struggle methods among ticks and tickborne diseases.

Keywords: tick, cattle, sheep, goat, Turkey





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Guilan, Iran; 20-22 May 2015 EPIDEMIOLOGICAL FEATURES OF PEDICULO- A SURVE

# SIS PREVALENCE IN MAZANDARAN PROVINCE

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The lice are small and wingless insects. Head, body and pubic lice are regarded as the ectoparasites in human. In addition to the direct bite, the body louse is able to transmit important diseases such as typhus, trench fever and relapsing fever. The research aimed to study pediculosis in Mazandaran University of Medical Sciences. Material & Method:In this descriptive study the data kept in the Health Department of Mazandarna Medical University was utilized. In order to address the issue, the data from 2006 until 2013 was analysed in terms of the total number of the infected cases, age and gender. The total number of infected cases from 2006 to 2013 was 105171: 2013 (N=19965), 2012 (N=17981), 2011 (N=18039), 2010 (N=15618), 2009 (13760), 2008 (N=10938), 2007 (N=5894) and 2006 (N=2932). The number of infected cases among female and male under 6 years was 2582 and 2315, respectively. The number of female and male between 6-10 years was 30465 and 7439, respectively. The number of infected female and male cases of 11-17 years was 25623 and 3510. The number of infected cases above 17 years was 30321 and 2916. 18184 of the participants had a previous history of i<sup>9</sup>nfection while 86987 of them did not. The number of cases infected with head, body and pubic lice was 105153, 11 and 7 respectively. The population of students infected with the disease was 92520. The number of the male and female participants, which were infected with the disease, was 14181 and 78339.From 2006 until 2013 there was an increasing trend in the rate of pediculosis infection. In 2006, 2.7 % and in 1392 18.9% were infected. In this 8-year study the highest number of infection was found among the female (84.6%) including (74%) female students. The rate of infection among the male was 15.4%, which is a lower rate in comparison with rate of the disease among the female. Therefore, educational programs should focus on the prevention and treatment methods of pediculosis (head lice) in schools since the population accounts for 87.9 percent of the infected cases. Education and treatment of students can be interpreted as the education and treatment of families and the education can be made available to the public through the mass media so that the spread of pediculosis can be controlled.

Keywords: epidemiologic, pediculosis, Mazandaran

## A SURVEY OF HARD TICKS (ACARI: IXODIDAE) ON HORSES IN NORTHWEST OF IRAN

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Ticks are one of the most important external parasites of horses, affecting horse industry with high economic impact. The main objective of this study was to determine the tick species of horses that occur in the Northwest of Iran as possible risk for tick-borne diseases. The study was conducted during the four seasons in 2012 in 25 villages which had a larger horse population in West Azerbaijan province. During four seasons a total of 877 Ixodidae ticks were collected comprising 4 genera of hard ticks including Hyalomma, Rhipicephalus, Boophilus and Dermacentor. These 4 genera included five species. The main species were Hyalomma anatolicum anatolicum, Hyalomma marginatum, Rhipicephalus bursa, Rhipicephalus (Boophilus) annulatus and Dermacentor marginatus. Although the parasite did occur throughout the year, there was a seasonal variation in the prevalence of ticks. Heavy tick infestations among horses pose a high risk of transmission of tick-borne disease to this livestock of the region. Therefore there is a need to investigate the potential tick vectors involved in the transmission of heamoparasites in horses in this region.

Keywords: tick, horse, West Azerbaijan, Iran.





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DETERMINATION THE FREQUENCY OF IX-ODID TICKS ON THE DONKEYS IN WEST AZER-BAIJAN PROVINCE, IRAN

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Donkeys (Equus asinus) are economical for use in small-scale farming and are handy in third world countries with a poor infrastructure. Ticks are one of the most important external parasites affecting the equine industry with high economic impact. The main objective of this study was to investigate the frequency of hard tick species (Acari: Ixodidae) on donkeys in West Azerbaijan province as possible risk for tickborne diseases. The study was conducted during the four seasons in 2012 in 15 villages of West Azerbaijan province. During four seasons a total of 455 Ixodidae ticks were collected from105 donkeys. The average number of ticks per donkey was 4.3. The most common tick species were Hyalomma anatolicum anatolicum, Hyalomma marginatum, and Rhipicephalus bursa. In this study, the highest number of identified and widely distributed tick species belonged to Hyalomma. Although the parasite did occur throughout the year, there was a seasonal variation in the prevalence of ticks.Some of the collected ticks may play an important role for transmission of vector borne diseases to donkeys; therefore, there is a need to investigate the potential tick vectors involved in the transmission of heamoparasites in donkeys in this region.

Keywords: tick, donkey, West Azerbaijan, Iran.

EPIDEMIOLOGICAL INVESTIGATION ON THE VISCERAL LEISHMANIASIS FROM GHIROKAR-ZIN COUNTY IN FARS PROVINCE, 2004-2014

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The World Health Organization has announced leishmaniasis as one of the most important parasitic diseases. The visceral form of the disease is called kalazar. Failure in treatment usually leads to death and even with treatment in 3 to 8% of the cases death will occur. Kalazar is a common disease of the Mediterranean area in man and animal and is placed in the Metazoonosis category. This disease is not highly prevalent in Iran, and have been reported from almost all provinces. This was a cross-sectional study of patients with a definite diagnosis of kala-azar. Information were obtained from hospitalized patients's file and data were analyzed using SPSS ver. 19. The findings of 25 patients with confirmed kala-azar from 2004-2014 showed that 4% of patients were Nomads, 56% Rural, and 40% were living in urban areas. 44% of the patients were female, and 56% were male. In terms of age, 86% were under one year, 72% in the age group 2-5 years, and 12% over 5 years old. The highest frequency was found in 2004 and the lowest in years 2011-2014. 96% of patients had complete recovery at discharge and 4% died. The most common clinical symptoms were prolonged fever, enlarged spleen and liver, anemia and weight loss, which is similar to other reports. The incidence of the disease in the study area is reduced. Due to the relatively high contamination in canids and their important role in the maintenance and spread of the disease, health interventions is recommended.

Keywords: leishmaniosis, kala-azar, Ghirokarzin





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# HUMAN DERMATITIS CAUSED BY OPHIONYS-SUS NATRICIS, A SNAKE MITE

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*Ophionyssus natricis* is a purely blood sucking parasite of snakes and is of worldwide distribution. Infected snakes often exhibit lethargy, pruritus, crusting dermatitis, and behavioral changes. *O. natricis* can also attack humans, causing popular vesiculo-bullous eruption of the skin. A 29 years old man working in a Zoo in Sari, Mazandaran, Iran, presented with itchy papullar eruption of the skin. He was noticed having small insects fixed on his skin and also large numbers of these same insects on a python and its cage in the zoo. Regarding to their morphological characteristics they were diagnosed as *O.natricis* (Geravis, 1844), a snake mite. It is the first report of *O. natricis* from Iran.

Keywords: Ophionyssus natricis, Python snake, dermatitis, Iran.

MORPHOMETRIC CHARACTERISTIC OF DIF-FERENT DEVELOPMENTAL STAGES OF DER-MACENTOR MARGINATUS UNDER LABORATO-RY CONDITIONS

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Dermacentor marginatus is a very common tick in the Mediterranean region and it can transmit various causative agents of diseases to animals and humans. This tick imposes a considerable economical burden annually. The present study was performed to study the morphometric characteristics and biology of different developmental stages of D. marginatus under laboratory conditions. D. marginatus ticks were acollected from sheep in Shahmirzad and suburb. The identification of D. marginatus was carried out by means of stereoscope and light microscope according to available systematic keys. Nourished female ticks weight and their length of body, capitulum and mouthparts were measured. After laying eggs and breeding, the weight of all developmental larva stages and the length of mouthparts were measured and recorded carefully. The mean of egg dimension was 566  $\times$  436  $\mu$ m. The lengthy of unfed larva body, hypostome and capitulum were  $690\pm10\mu m$ ,  $75\pm5 \mu m$  and  $172\pm7 \mu m$ , respectively. The weight of egg was calculated 0.05 mg and the weight of unfed larva, nymph and female were 0.02 mg, 0.14 mg and 4.66 mg, respectively; whereas the weight of replete larva, nymph and female were recorded 0.5 mg, 11 mg and 380 mg, respectively. Moreover, the length of unfed nymph, hypostome and capitulum were recorded 1300±50µm, 135±5 µm and 280±10 µm, respectively. The longest length and width in replete female were observed  $8.4 \times 12.6$  mm. Conclusions: The current investigation presents new information on biology of D. marginatus under standard laboratory conditions. In addition, investigation on ticks under laboratory conditions increases our knowledge regarding their biology and potential risks.

Keywords: morphology, biology, life cycle, tick, laboratory condition, Iran





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## CYPERMETHRIN RESISTANCE IN RHIPICEPH-ALUS (BOOPHILUS) ANNULATUS TICKS IN NOOR TOWNSHIP, NORTH OF IRAN

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Rhipicephalus (Boophilus) annulatus (R. (B.) annulatus) is a major pest of domestic ruminants in North of Iran. The use of chemical acaricides is at the center of the national tick control strategy, however, pesticide resistance has led to operational failure. The aim of this study was to determine the susceptibility status of this species to common cypermethrin acaricides in Noor Township, North of Iran. A multistage cluster random sampling method was used to examine 823 domestic animals (cattle, sheep and goats) in three different geographical areas of plain/littoral, jungle and mountains. After species identification using appropriate keys, the fully engorged R. (B.) annulatus were kept in an insectary under 27  $\pm$  2°C temperature, 78  $\pm$  5% relative humidity and 12:12 photoperiod until they laid eggs in about 4-10 days. Seventeen populations of R. (B.) annulatus ticks were tested with cypermethrin. The two-week old larvae were used for the Larval Packet Test (LPT) with Whatman # 541 filter paper treated with the serial discriminating doses of cypermethrin. The mortality of the treatment and control replicates was scored after 24 hours. Probit analysis was employed to calculate the LC50 and LC99 of the populations and ANOVA-Tukey test was also used to compare the probit analysis data between populations. Population 75 showed a resistance ratio of 129 with cypermethrin when compared to the most susceptible population 23 at the LC99 level which is about 75-fold higher than the dose recommended by the formulating company. We documented for the first time a R. (B.) annulatus population in Iran with resistance to cypermethrin insecticide. Conclusions: The resistance ratio of insecticide tested confirms operational failure with cypermethrin. Therefore monitoring the acaricide susceptibility status of the filed populations is the key to manage insecticide resistance by implementing resistance management strategies in the long run and also choosing alternative acaricides for the short term.

Keywords: cypermethrin, insecticide resistance, larval packet test, pyrethroid, *Rhipicephalus (Boophilus) annulatus*, tick CARVACROL AS A NOVEL PLANT DERIVED IN-SECTICIDE, EFFECTIVE AGAINST CIMEX LEC-TULARIUS L.

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The common bed bug, Cimex lectularius, is a nocturnal bloodsucking ectoparasite of humans. It is a pest causing serious discomfort for people living in infested houses. The most widely used insecticides against bed bugs are the pyrethroids and the organophosphates. Resistance against pyrethroids and organophosphates have been reported from several parts of the world. Carvacrol is a monoterpene phenol that occurs in essential oils of the family Labiatae. It was reported to have insecticidal activity against agricultural pests. With the above in mind, the aim of the current study was to assess the efficacy of carvacrol against Cimex lectularius. Contact bioassay was done to evaluate the efficacy of carvacrol in comparison to permethrin against Cimex lectularius. For this purpose, filter papers were treated with different concentrations of carvacrol and permethrin (1, 2, 4, 8 and 16 µl) in a total volume of 50 µl of ethanol. Each treated paper after drying was placed on the bottom of a petri dish and 10 adult mites were introduced into them then sealed with another lid and wrapped with parafilm. After exposure time of 24 hour, bugs were considered as dead if they exhibited no movement after being prodded with a pin. In 0.02% concentration all of the bed bugs were alive in both carvacrol and permethrin groups. In the concentration of 0.04%, mortality rate were 80% in carvacrol treated groups while permethrin caused no mortality. In concentrations of 0.08%, 0.16% and 0.32% carvacrol mortality rate were 100%. Surprisingly permethrin in all of the concentrations did not kill any bed bugs. The survey showed that permethrin-resistance was high in bed bugs.Based on the promising efficacies seen with carvacrol in the current work, this plant-derived product can be suggested as a novel agent for pest management.

Keywords: Cimex lectularius L, carvacrol, permethrin, acaricide





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## FRIST REPORT OF HYALOMMA AEGYPTIUM IN TORTOISE IN SOUTH KHORASAN PROVINCE

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A two years old female tortoise was killed in a road accident by a truck in Shosf County around Nehbandan City in South KhorasanProvince, southeast of Iran. In inspection of surface body four ticks was observed. Ticks were collected with forceps and fixed in 70% ethanol. The collected ticks were brought to the parasitology research laboratory of Shahid Bahonar University, Kerman. The ticks were collected in this case were identified as *Hyalomma aegyptium*. It is the first report of Hyalomma aegyptium of tortoise in Shosf County around Nehbandan City in South Khorasan province, southeast of Iran.

Keywords: Hyalomma aegyptium, tortoise, Nehbandan

#### STUDY OF ECTOPARASITES ON STRAY DOGS IN MAZANDARAN, GUILAN AND QAZVIN PROV-INCES

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Ectoparasites play an important role not only as pests but also as vectors of various infectious diseases of humans, livestock, pets and wild animals. The aim of this survey was to determine the prevalence of ectoparasite infestations in stray dogs in Mazandaran, Guilan and Qazvin provinces from December to March 2013. Seventy dogs from studied areas (30, 20 and 20 dogs from Mazandaran, Guilan and Qazvin province, respectively) were examined for ectoparasite infestation. In order to survey on mites, suspected areas to dermatitis were scraped deeply with an oily scalpel blade. Deep otic swab specimens were obtained from all dogs for detection of ear mite. The rate of infestation in these areas was 100%, 68.5% and 93.3%, respectively. In this study, the most common ectoparasites on dogs were flea, lice, ticks, flies and mites, respectively. The isolated arthropods were fleas in 45 (77.5%), lice in 29 (50%), ticks in 5 (8.6%), flies in 4 (6.8%) and mites in 3 (5.1%) infested dogs. The ectoparasite of the dogs included 4 flea species, Ctenocephalides canis (29.8%), C. felis (19.9%), Pulex irritans (2.9%) and Xenopsiella cheopis (0.7%), 1 louse species, Trichodectes canis (41.3%), 1 tick species, Rhipicephalus sanguinus (0.7%), 1 fly species, Hippobosca sp. (1.1%) and 1 mite species, Sarcoptes scabiei (3.6%). Triple and double infestation was observed in 8.6% and 39.7% of the infested dogs and 51.8% presented single infestation with lice or fleas. The highest prevalence of infestation was observed in < 3years old (88%) followed by 3-5 (77.7%) and > 5 years old (70.5%). Ectoparasitic infestations in male and female dogs were 33 male (89.1%) and 25 female dogs (75.7%). No statically difference was observed between ectoparasite infestation and dog's age and gender (p>0.05). Conlusion: According to the high ectoparasite infestation of stray dogs in this study and their transmission risks to humans and other animals, regular monitoring of distribution patterns of ectoparasites is an important concern to control the arthropods and arthropods-borne diseases.

Keywords: stray dogs, ectoparasites, parasitic arthropods





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ISOLATION AND IDENTIFICATION OF PATHO-GENIC BACTERIA POSSIBLY TRANSMITTED BY MUSCA DOMESTICA IN THE REGION OF SARI, IRAN

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The house-fly, Musca domestica L., acts as an important mechanical vector for transporting lots of pathogenic microorganism agents, such as: bacteria, parasites, fungi and viruses. The aim of this study was to determine the bacterial contaminations of houseflies found around various garbage of Sari, Iran in 2013. In this study totally 2020 houseflies were collected to isolate their bacteria, from three sites of Sari, including slaughterhouses, city and rural waste. Both the external surface and digestive system of the collected flies were investigated for the presence of bacteria. Bacteria from fly sample were isolated and Identified using the normal and standard techniques such as culture methods, gram staining and biochemical tests. This study showed that 8 species of bacteria include Staphylococcus sp., Streptococcus sp., Citrobacter.sp sp., Enterococcus sp., Enterobacter sp., Proteus sp., Escherichia sp., and Klebsiella sp. were isolated from external surface and digestive system of Musca domestica. The most common isolated bacteria were Escherichia coli (19.7%). Conclusions: This study showed that flies can spread pathogenic microorganisms in human habitats, directly or as a vector. Thus, biosecurity plans and control programs are important to prevent diseases that may transmit by contaminated flies.

Keywords: Musca domestica, pathogenic bacteria, Sari

ISOLATION OF MEDICALLY IMPORTANT FUNGI FROM COCKROACHES TRAPPED AT HOSPITALS OF SARI, IRAN

## <u>Farzad Motevali Haghi</u><sup>1</sup>, Reza Aghili<sup>2</sup>, Shirzad Gholami<sup>3</sup>, Bahar Salmanian<sup>4</sup>, Hasan Nikookar<sup>5</sup>, Maryam Khangolzadeh Gerav<sup>6</sup>, Masumeh Eslamifar<sup>7</sup>, Hosna Hajati<sup>8</sup>

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This study was done to evaluate the presence of medical important fungi on the external surface of cockroaches collected from the public and residential areas of three hospitals of Sari, Iran. Materials & Mthods: A total of 38 cockroaches were caught from staff resting rooms / working areas, the ward floors and patient rooms, during two consecutive days at three hospitals between December and November, 2013. The frequency of Blattella germanica and Periplaneta americana among 38 trapped cockroaches were 84.2 and 15.8 percent, respectively. Candida spp. was the most yeast isolated (94.7%) on external surfaces of cockroaches and Rhodotrula spp. (57.9%) was the next. Also, Aspergillus spp. (84.2%), Fusarium spp. (15.8%), Penicillium spp. (10.6%) and Geotrichum spp. (10.6%) were the most molds appeared on external surfaces of cockroaches. Among 36 (94.7%) cockroaches, 4 species of Candida were identified by mycological examinations. C. glabrata (52.8%) and C. albicans (38.8%) were the highest species isolated from cockroaches. A. niger (50%) was the most species that was isolated from cockroaches.Cockroaches are vectors of microbial agents such as fungi, yeast, etc. that can cause nosocomial infections. Thus, public centers such as hospitals should have definite plan to combat with these pest insects.

Keywords: fungi, cockroach, nosocomial infection





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ANOPHELINE LARVAE COMPOSITION (DIP-TERA: CULICIDAE) IN PISHIN REGION, SARBAZ DISTRICT, SOUTHEASTERN IRAN

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## Sarbaz district with indigenous malaria cases located in Sistan and Baluchistan Province, southeast of Iran. The objective of this study was to determine the fauna and the species composition of Anopheline Larvae for future mosquito control programs. Fieldwork was conducted in the Pishin region as a main malarious area located in the Sarbaz district. Anopheline larvae (Diptera: Culicidae) were collected by dipping technique during 2012-2013. Breeding sites including 20 permanent and non-permanent habitats were checked twice a month. The preserved and mounted larvae were identified using morphological standard keys. In this study, 2150 Anopheline larva belonged to 4 species were collected: A.culicifacies s.l Giles, A. stephensi Liston, A. superpictus Grassi and A. subpictus Grassi. A. stephensi (933) 43.4% and then A.culicifacies s.l (629) 29.3% had the most abundance, respectively. In permanent breeding sites, A. culicifacies and in non vegetation habitats A. stephensi had the highest abundance. Also A. subpictus was observed in permanent, natural, clear and sunny habitats. Also, A. superpictus was collected in the mentioned habitats but it preferred non vegetation sites. Overall, The highest abundant was recorded in April, June, September and November. The increase of stagnant water due to the Seasonal and Manson variations were significantly associated with the presence of anopheline larvae with a higher density. The rainfall and stagnant water, as well as the Manson variations, could influence the weather of this area significantly and these subjects can support the persistence of malaria vectors in this region. It is suggested that regular monitoring of anopheline larvae, required in malaria elimination program especially in evaluation of larval control methods, should be performed.

Keywords: malaria, anopheline, Sarbaz, Iran

EVALUATION OF BACTERIAL INFECTION AND ANTIBIOTIC SUSCEPTIBILITY OF THE BACTE-RIA ISOLATED FROM COCKROACHES IN EDU-CATIONAL HOSPITALS OF MAZANDARAN

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This study was done to investigate the bacterial infection and antibiotic susceptibility of the bacteria isolated from cockroaches in educational hospitals of Mazandaran University of Medical Sciences in 2013. In this descriptive cross-sectional study, cockroaches of 4 hospitals were caught via direct collection in a 45 days period. Medically important bacteria were isolated from their outer surface and digestive tract by standard procedures and antibiogram test was done using different antibiotics.Culturing the supernatant of outer surface wash and the digestive tract of cockroaches resulted in the separation of Proteus mirabilis, Klebsiella pneumonia, E. coli, Enterobacter, Pseudomonas aeruginosa, Acinetobacter baumannii, Staphylococcus aureus, Staphylococcus epidermidis. The main common bacteria were Proteus mirabilis, Klebsiella pneumonia in three hospitals, and Pseudomonas aeruginosa, Acinetobacter baumannii in one hospital. Staphylococcus epidermidis isolated from different hospitals had the highest susceptibility to various antibiotics, however, Acinetobacter baumannii had the lowest susceptibility to different antibiotics. Conclusions: Cockroaches can carry pathogenic bacteria in hospitals and they can cause hospital-acquired infections. Regarding to their potential in drug-resistant pathogens transmission, periodic fumigation and sanitation programs of the hospitals would be emphasized to combat these insects in the hospitals.

Keywords: cockroaches, pathogenic bacteria, nosocomial infection





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PREVALENCE OF ECTOPARASITES IN SHEEP AND GOATS IN KAMYARAN KURDISTAN)

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Parasitic infections are one of the parameters of decline in production of livestock causing serious economic loss to farmers, the tanning industry, and the country as a whole. Due to the importance of sheep and goats tanning industry in Kamyaran city, Kurdistan province, an investigation was conducted on ectoparasites of these livestock for the first time. Out of a total of 256 sheep and goats from 32 villages, 1500 samples were collected from the slaughterhouse of Kamyaran, then the samples were examined for ectoparasites in parasitology laboratory of Faculty of Para Veterinary Science. Cutaneus myiasis was only Przhevalskiana larvae, the tick species identified in sheep, and goats were Rhipicephallus and Boophillus. Ctenocephalides and Pulex fleas reported 50% and 42% in the ewe under 1 year and ewe over 1 year, respectively. Also, the lices such as Damallina, Lingognathus and Heamathopinus were reported. Lucillia coaprina larvae were found in skin lesions in sheep (80%) and Oestrus ovis larvae were seen in skin lesions and sinus tracts of goats and sheep.In this study, among the lowlands, foothills and mountainous regions, the highest rate of infestation were reported in the lowlands (90%).

Keywords: livestock, ectoparasites, sheep, goats, Kurdistan

APPLICATION OF FASCIOLA HEPATICA FRAC-TIONATED PROTEINS FOR SERODIAGNOSIS

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Diagnosis of human fascioliasis is performed with stool examination as a gold standard that is not effectient and shows poor sensitivity during the acute and chronic phase of infection. Recently, serodiagnosis via specific antigens has become an excellent alternative method to coprological examinations. In this study, Fasciola hepatica excretory/secretory (ES) antigens were fractionated by fast protein liquid chromatography (FPLC) and applied for serodiagnosis of fascioliasis. Excretory/secretory antigens of F. hepatica were collected by incubating the adult parasites in RPMI for 24 hours. The ES proteins were separated by anion exchange chromatography and AC-TA prime plus FPLC. The peak fractions were evaluated by SDS-PAGE and silver staining. The coated fractions in ELI-SA plates were examined with the sera of 6 fascioliasis patients and 16 healthy people as positive and negative controls, respectively. Moreover, to study the cross reaction of antibodies from different parasite infections, we applied 4 strongyloidiasis and 4 hydatidosis patients' sera, simultaneously. The results were analyzed statistically. Seven protein fractions were collected by anion exchange chromatography. Most of them eluted when the elution buffer contained 60 -70 mM NaCl. One of the fractions contained a single protein band, but the others showed 2-5 protein bands in electrophoresis. Some of the fractions could better differentiate Fasciolais patients' serum, in comparison to crude extract and showed no cross reactivity with antibodies raised against Strongyloides stercoralis.Our study showed that FPLC is a suitable, economical, reproducible, and rapid method for fractionation and purification of F. hepatica immunogens. Some of the fractions are reliable for serodiagnosis of human fasioliasis and could differentiate fasciolosis from similar parasite infections.

**Keywords:** FPLC, excretory- secretory proteins, fascioliasis, fractionation, ELISA





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## PREVALENCE OF FASCIOLA SPP. INFECTION IN LIVESTOCKS BY STOOL EXAMINATION IN DASHT ROOM COUNTY OF YASUJ IN SPRING AND SUMMER 2013

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According to previous human serologic studies on slaughtered livestock suggesting Fasciola infection as a new emerging focus in this region, this study was performed to evaluate the prevalence of Fasciola spp. infection in livestock in Dasht Room County by stool exam in 2013. In this cross-sectional study, 600 stool samples of animals including sheep, goats and cattle were collected from six villages in Dasht Room region, Boyer Ahmad district, and examined by standard formalin- ether precipitation method. 174 out of 600 stool samples (29%) were positive for Fasciola eggs, including 63 sheep (26.03%), 40 goats (23.3%) and 71 cattles (37.9%). Significant differences between the infection rates of livestock were not observed in spring and summer. The highest and lowest infection was observed in Tangary and Mansour Abad villages respectively with statistically significant differences (P < 0.05). The high and low infection was observed in cattle and goat. Statistically significant difference was observed between them in summer (P < 0.05). Conclusions: According to seropositivity of human fasciolosis in this region based on study of Sarkari et al, 2012, and high contamination of livestock in the present study (29%), Dasht Room County must to be considered as a potentially high risk area for fasciolosis.

Keywords: Fasciola, livestock, infection, Dasht Room, Yasuj, Iran

# PATTERN OF HUMAN FASCIOLIASIS IN THE PROVINCE OF GIULAN, IRAN (2008-2014)

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Fascioliasis is caused by two species of parasitic trematode that mainly affect the liver. It belongs to the group of foodborne trematode infections and is a Zoonoses. Human cases are now increasingly reported from Europe, the Americas (where only F. hepatica is transmitted) and from Africa and Asia (where the two species overlap). WHO estimates that at least 2.4 million people are infected in more than 60 countries all around the world. It is estimated that at least 6 million people are at risk of the infection in Iran. Giulan province is the main endemic area in Iran and even in Asia with most cases reported from Bandar-Anzali district. The aim of this study was to review the pattern of the disease in Guilan province during 2008-2014. This study was an analyticdescriptive and manner descriptive of retrospective study. All cases of human fascioliasis documented in Giulan Central Health Service during 2008 to 2014 and reported. A total of 375 confirmed reported fascioliasis patients were studied, of them 245 patients (65.2%) was urban and 130 (34.8%) were living in rural areas, the difference was significant (P < 0.05). Bandar-Anzali city with 169 cases (45.1%), Rasht, with 77 cases (20.5%) and Lahijan with 70 cases (18.7%) were the most infected areas and in other cities Fasciola prevalence was low and the difference was significant (P <0.05). Most patients was female with 246 cases (56.6%) while 129 (34.2%) patients were males and the difference between sexes was significant (P <0.05). The highest number of the cases were in the age group 59-40 years, 141 (37.6%), and the lowest rate was related to age group 0-19 years, with 30 cases (8%) and no significant difference (P> 0.05) was seen. In addition, Fascioliasis showed no significance according to job, education and season (P> 0.05). The results show that fascioliasis is endemic in Guilan province and transmission of the disease is continuously occur.

Keywords: fascioliasis, Giulan, Iran, Anzali.





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## THE PREVALENCE OF LIVER TREMATODES IN SLAUGHTERED LIVESTOCK IN KAZERUN AB-ATTOIR, SOUTH OF IRAN

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Introdction & Objectives: Fasciola hepatica and Dicrocoelium dendriticum, are recognized as the most important helminthic parasites of sheep, causing considerable economic losses to ranchers due to reductions in milk and meat production, condemnation of parasitized livers, abortion, increased mortality, and the expense of control measures. The main enzootic area of sheep liver trematodes lies in the north of Iran, but Fasciola spp. and D. dendriticum also occur in the northwest region of Iran. This study was designed to estimate the prevalence of zoonotic trematodes F. hepatica and D. dendriticum infection among sheep, cattle and goat in Kazerun abattoir, Fars province, south of Iran, from March 2011 to January 2013. In this retrospective study, a total of 12381 sheep, 6473 cattle and 22847 goats have been studied considering liver infection with Fasciola spp. and Dicrocoelium dendriticum in Kazerun abattoir. Prevalence rate of Fasciola spp. in slaughtered sheep, cattle and goats has determined 41 (0.33%), 107 (1.65%) and 56 (0.24%), respectively and infection with D. dendriticum has only observed in goats 1(0.004%). Considering the reduction of livestock infection rate with liver trematodes in this study in comparison with previous studies in our country, it is necessary to find the responsible agents in various climates and conditions.

Keywords: Fasciola, Dicrocoelium dendriticum, enzootic, Kazerun, Iran.

## SONOGRAPHY AND CT SCAN APPLICATION IN DIAGNOSIS AND TREATMENT FOLLOW UP OF FASCIOLOSIS: A CASE REPORT

Shahla Jahan Bin<sup>1</sup>, Farshad Hoseyni<sup>2</sup>, Urang Ilami<sup>3</sup>, Abdolali Moshfe<sup>4</sup>, Bahador Shahryari<sup>5,</sup> Saeed Jokar<sup>6</sup> 1- Assistant Professor, Department of Radiology, Shahid Beheshti Hospital, Yasuj, Iran. \* 2- Student, Department of Radiology, Imam Sajjad Hospital, Yasuj, Iran. farshadhoseyni9@gmail.com 3- Associate Professor, Yasuj University of Medical Sciences, Yasuj, Iran. 4- Associate Professor, Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, Iran. 5- Professor, Faculty of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran. 6- General Physician, School of Medicine, Yasuj University of Medical Sciences, Yasuj, Iran. This is the first case report of fasciolosis from Yasuj diagnosed by sonography and CT scan. The patient is a 15 y/o male referred to Yasuj Shahid Beheshti hospital because of weight loss from 3 years and intermittent fever from 1 year ago. His blood index and liver function test showed considerable changes. In abdominal sonograghy, two hypoechoic areas in RT liver lobe were detected. No vascular flow is detected during color Doppler study, intra and extra hepatic bile ducts appeared normal. The abdominal CT scan with and without contrast showed multiple hypo attenuated lesion at anterior segment of right liver lobe. Faint peripheral contrast enhancement of the lesion observed. According to sonography and CT scan finding, he is susceptible to fasciolosis. Finally, serologic ELISA test was positive for Fasciola hepatica antibodies and confirmed the disease. After 3 months post treatment with triclabendazole, the CT images and sonography without contrast revealed that the lesions were completely disappeared and after contrast liver had normal pattern. Since the serologic tset is not available in some part of Iran, CT scan and sonography can be used for diagnosis of fasciolosis based on clinical symptoms and changes on blood indexes and these methods can be used for follow up treat-

Keywords: fasciolosis, sonography, CT scan, diagnosis

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Guilan, Iran; 20-22 May 2015

# A SLAUGHTERHOUSE STUDY ON PREVALENCE OF SOME ZOONOTIC HELMINTHES IN CATTLE IN ALBORZ PROVINCE, IRAN

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The aim of study was to determine the prevalence of helminthic parasite in cattle, and its relation with climatic indicators in Alborz province, Iran. Hydatidosis causes product reduction moreover, remarkable entanglements for human while liver distomatosis lead to liver damage, weight loss in animal and malignancy like cancer in man so, they are important in terms of economic and public health. In a cross sectional study the rate of prevalence of hydatic cyst, fasciolosis and dicrocoeliasis in slaughtered cattle has studied from January 2009 to Mars 2013. The data were collected by archival reports from veterinary organization and analyzed by SPSS software. In 131668 slaughtered cattle, 5 year prevalence of fasciolosis infection rate is 2.09%, Dicrocoelium dendriticum 2.46% and hydatid cyst 9.95%, respectively. There was a direct correlation and significant statistic relationship between hydatid cyst prevalence and moisture in spring (p=0.023, r=0.928) and a reverse correlation and significant statistic relationship with temperature in spring (p=0.004, r=-0.978) moreover, There was a direct correlation and significant statistic relationship between fasciolosis prevalence and amount of moisture in spring in cattle (p= 0.026, r= 0.922) and a reverse correlation and significant statistic relationship between dicrocoeliasis prevalence and temperature in spring (p= 0.048, r= -0.881). These results can be used in control and prevention program in future by authorities and executive planners to help dairy and leather industries in decreasing the losses as well as reducing accidental infections risk

Keywords: hydatic cyst, fasciolosis, dicrocoeliasis, slaughterhouse

# TREND OF FASCIOLIASIS IN MAZANDARAN PROVINCE

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The disease caused by the parasite Fasciola hepatica is called fascioliasis or distomiasis. The parasite is prevalent among the herbivores and is widespread in the world. Human infection usually follows consumption of aquatic plants such as watercress and wild mint or metacercariae carrying water. The present study aimed to investigate the cases of infection among human population in Mazandaran from 1999 to 2013. The study was descriptive and used the data kept in the Health Department of Mazandarna University of Medical Sciences. The data was analyzed in terms of the participants' gender, age and location. All the patients were received Egaten following definitive diagnosis. From 1378 to 1392 the total number of individuals who had been infected with fascioliasis was 136 cases, including 134 (98.5%) cases in Nowshahr, Challus, Tonekabon and Ramsar which are located in the western parts of the province. Two cases (1.5%) were reported in Sari and Babolsar. All the infected cases were treated with Egaten and cured completely. In none of the cases treatment failure or drug resistance were observed.Consumption of raw vegetables has become customary in Mazandaran and olive with the walnut paste preppard with wild vegetables are common extensively in cities such as Nowshahr, Challus, Tonekabon and Ramsar which are all located in the western parts of the province. The situation accounts for 98.5% of the infected cases. Therefore, the spread of the disease should be controlled by providing health education regarding methods of fascioliasis prevention through washing and deparasiting of raw vegetables especially the wild ones in addition to avoiding consumption local tastes which are traditionally prepared by local vegetables.

Keywords: fascioliasis, Fasciola hepatica, Mazandaran





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# PREVALENCE OF FASCIOLA IN CAMELS (CAMELUS DROMEDARIES) SLAUGHTERED AT INDUSTRIAL MASHHAD ABATTOIR OF KHORA-SAN PROVINCE

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Hepatic fascioliasis is a zoonotic disease that affects the bile ducts of ruminants, pigs, horses, rabbits and other herbivores, as well as humans. Few studies have been conducted on the incidence and control of the parasites of camels. The liver of 1000 native camels slaughtered at Mashhad industrial abattoir was examined for *Fasciola* in summer 2014-2015. Our findings revealed 50 of the examined livers harboured *Fasciola hepatica*. Although 91 *Fasciola* were collected from a camel, the average number of *Fasciola* was 15. Most of the infected camels were brought from valleys of Torbat and Neyshabour cities. It is concluded that camel should be considered as a host for *Fasciola*, which can play a major role in the dessimination of liver fluke infection to other herbivoures as well as human beings.

Keywords: camel, Fasciola hepatica, Mashhad

DETECTION OF FASCIOLA HEPATICA AND FASCIOLA GIGANTICA COMMON AND UNCOM-MON ANTIGENS, USING RABBIT HYPER IM-MUNE SERUM RAISED AGAINST THEIR EXCRE-TORY-SECRETORY AND SOMATIC ANTIGENS

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Fasciolosis is an important neglected helminth disease caused by two liver fluke, Fasciola hepatica and Fasciola gigantica. The two species of Fasciola are usually different in their morphological and molecular features. They have also common and uncommon antigens in both their somatic and excretory secretory metabolites. In this study, we compared somatic and excretory-secretory (ES) antigens of F. hepatica and F. gigantica, by using rabbit hyper immune serum raised against these antigens. Adult flukes were collected from the bile ducts of infected animals and species of the fluke was confirmed by RFLP-PCR. ES and somatic antigens of both species were prepared. Rabbits were subcutaneously immunized with either ES or somatic antigens to produce antibodies against these antigens. F. hepatica and F. gigantica ES and somatic antigens were separated by SDS-PAGE and evaluated by western blotting, using rabbit hyper immune serum raised against each antigen. Result: SDS-PAGE pattern of F. hepatica and F. gigantica somatic antigens was similar and both of them revealed 30 protein bands, ranging from 18 to 180 kDa. In contrast, SDS-PAGE patterns of ES antigen of the two species were different. While protein bands with molecular weight of 18, 27, 29, 48, and 62 kDa were common in both species, bands of 19, 45, 55 and 58 kDa were only noticed in F. hepatica ES antigen. Rabbit polyclonal antibodies, raised against F. hepatica and F. gigantica ES antigen, reacted with main five protein bands, 25, 27, 29, 62 and 67 kDa and polyclonal antibodies raised against somatic antigens of both species reacted with 3 protein bands, 25, 27 and 72 kDa.findings of this study revealed that the common 25, 27 and 29 kDa protein bands are present in both species of Fasciola and might be considered for serodiagnosis of fasciolosis. Moreover, bands of 62 and 67 kDa in ES antigen and 72 kDa in somatic antigens of both species were immunodominant and might be suitable candidates for development of serological assays for diagnosis of fasciolosis.

Keywords: western blotting, *Fasciola*, somatic antigen, excretory-secretory antigen





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GENOTYPING AND PHYLOGENETIC ANALYSIS OF FASCIOLA SPP. ISOLATED FROM SHEEP AND CATTLE USING PCR-RFLP IN ARDABIL PROV-INCE, NORTHWESTERN IRAN

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The aim of this study was to detect the genotype of Fasciola spp. in Meshkin-Shahr, Ardabil province, northwestern Iran in different hosts using PCR-RFLP. The parasite hosts included cattle, and sheep. Overall, 70 adult flukes from livers of slaughtered animals were collected from the abattoirs of mentioned areas. They included 35 samples from infected sheep and 35 samples from 35 infected cattle. PCR-RFLP and sequence analysis of the first nuclear ribosomal internal transcribed spacer (ITS 1) region from Fasciola species were used to conduct the study. The fragment of approximately 700bp in all of the Fasciola samples was amplified. PCR products of ITS 1 were subjected for digestion by restriction enzyme. RsaI restriction enzyme was selected for RFLP method that caused the separation specifically of Fasciola species. Amplicons with the sequences of F. hepatica had a pattern of about 360, 100, and 60 bp band size, whereas F. gigantica worms had a profile of 360, 170, and 60 bp in size, respectively. Results based on PCR-RFLP analysis were confirmed by sequence analysis of representative ITS 1 amplicons. No hybrid forms were detected in the present study. All sheep were infected with F. hepatica but cattle were infected with both species. Both species of Fasciola are present in Ardabil. The method described here can be valuable for identification of Fasciola species in endemic areas, regions with intermediate species and in that overlapping distribution area.

Keywords: Fasciola, genotyping, fasciolosis, PCR, Iran

## MOLECULAR IDENTIFICATION OF FASCIOLA HEPATICA INFECTION IN GALBA TRUNCATULA IN SOUTH - EAST OF IRAN

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Fasciola hepatica is a digenean trematode with a worldwide distribution causing fasciolosis. The disease affects humans and domestic ruminants and causes important medical and economic losses. Fascioliasis is endemic in 61 countries including Iran and the number of people at risk exceeds 180 million. Freshwater lymnaeid snails have been known to play an important role as intermediate host of Fasciola species. At least 20 species of the snails including Galba truncatula have been potentially involved in Fascioliasis transmission. In the southeast of the country, there are no data on the status F. hepatica infection in the snail intermediate hosts. The purpose of the present study was to determine the Galba truncatula infection with F. hepatica in southeastern regions of Iran. Galba truncatula specimens were collected according to the shell morphological characteristics from 14 habitats in Kerman province in southeastern Iran. Geographical characteristics of each region were recorded in a GPS device. The snail species identified by the PCR amplification of partial Cox- 1 and ITS-2 genes. F. hepatica infectin of the snails were detected using PCR, amplification of a 124 bp non-coding tandem repeat found in Fasciola genome. No cross-reactions occurred with DNA of either of the trematodes or snails. Galba truncatula was isolated from Jiroft, Bardsir, Faryab, Pariz, Deh-Bakri and Deh-e Lulu regions in Kerman province. G. truncatula isolates collected from nine out of 14 habitats were infected with F. hepatica. This study presents the first molecular evidence of F. hepatica infection of G. truncatula in southeastern Iran. F. hepatica life cycle is perpetuated between G. truncatula and domestic ruminants in the region. This is the first molecular study in the south east of Iran to identify the intermediate host of Fasciola hepatica. This study showed Galba truncatula as intermediate hosts of F. hepatica in the south of Iran, similar to that of Guilan province.

Keywords: Fasciola hepatica, Galba truncatula, Kerman, Iran





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PREVALENCE OF HUMAN FASCIOLOSIS AND INTESTINAL HELMINTHES IN RURAL AREAS OF BOYERAHMAD TOWNSHIP IN KOHGILUYEH AND BOYERAHMAD PROVINCE IN 2014

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Fasciolosis caused by two species of the Genus Fasciola, Fasciola hepatica and Fasciola gigantica. Disease is common in animals in Boyerahmad Township and this area is considered as a new focus of human fasciolosis. On the other hand, rate of infection with intestinal helminthes in this township is unknown. This study aimed to determine the prevalence of fasciolosis and intestinal helminthes in inhabitants of rural areas of Boyeramad in the Kohgiluveh and Boyerahmad province in 2014. Stool samples (1025) were collected from inhabitant of 50 randomly selected villages in Boyerahmad Township. Samples were evaluated with modified Teleman and formalin ethyl acetate methods for detection of helminthes ova. Blood samples were collected from Fasciola positive cases and assessed with ELISA and Western blotting. DNA was extracted from Fasciola eggs from stool of positive individuals and evaluated by molecular (PCR) method. In addition, the PCR products were sequenced. Out of 1025 cases, 473 (46.1%) were male and 552 (53.9%) were female. The mean age of the subjects was 20.25 years (1-89 years). Among the studied subjects, Fasciola eggs were detected in stools of two cases. Their infection was confirmed by repeated tests. Blood samples were obtained from the Fasciola positive cases and their infection was confirmed by ELISA and Western blotting. PCR and sequencing revealed that both cases were infected with Fasciola hepatica. In this study, seven patients (0.68%) were found to be infected with Hymenolepis nana, 4 cases (0.39%) with Enterobius vermicularis, and one case (0.09%) with Trichuris trichiura. Findings of this study showed that Boyerahmad district is one of the endemic areas of human fasciolosis in which control and prevention programs should be considered. In addition, rate of helminthic infections in rural areas of the district has drastically declined over the past years.

Keywords: fasciolosis, prevalence, helminth infection, Boyerahmad

#### PERFORMANCE OF AN INDIRECT ELISA FOR SEROLOGICAL DIAGNOSIS OF HUMAN FASCIO-LIASIS

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Human fascioliasis is an emerging helminthic zoonotic disease in some areas of the world, including Iran. Serological diagnosis, based on antigenic fractions of the parasite can be used for the early diagnosis of the infection. The current study aimed to evaluate the performance of an indirect ELI-SA system for serological diagnosis of human fascioliasis. A 27 KDa immunodominant antigen of Fasciola hepatica somatic antigens was purified from the SDS-PAGE gel and evaluated by enzyme-linked immunosorbent assay (ELISA) with sera samples of human fascioliasis patients, healthy controls and patients with other parasitic infections. Of 15 sera of fascioliasis patients, all (100 %) were found to be positive by ELI-SA while only 4 cases (6.25%) of non-fascioliasis patients were false-positive by this system. Sera from healthy controls did not react with the antigen and were all negative. Accordingly, the sensitivity and specificity of the test were 100% (95% CI: 84-100%) and 93.6% (95% CI: 83-97%), respectively.Findings of this study demonstrated that the indirect ELI-SA, based on the 27 KDa subunit of F. hepatica somatic antigens, is a reliable method for serodiagnosis of human fascioliasis.

Keywords: fascioliasis, immunodiagnosis, ELISA





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# TRICLABENDAZOLE EFFECTS ON THE EXCRE-TORY-SECRETORY PROTEOME OF FASCIOLA HEPATICA IN TWO DIMENSION GEL ELEC-TROPHORESIS

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Fasciola hepatica and F. gigantica are digenetic trematodes that cause fascioliasis. The aim of this study was to evaluate the protein spots of excretory- secretory products of Fasciola hepatica using two dimension electrophoresis method in the presence and absence of triclabendazole drug which can be considered to detect the target protein of the drug. F. hepatica parasites were collected from infected cattle livers, divided in two groups and cultivated in RPMI 1640 medium. First group treated with triclabendazole (TCBZ) and second group considered as control. The excretory-secretory (ES) products of each group were separated and total protein determined by Bradford method. To provide proteome spots, the ES proteins were precipitated and two dimension electrophoresis (2-DE) gel prepared. Protein amounts of two groups were compared using the statistical t-test and protein spots from 2-DE in test and control groups were also statistically analyzed. The protein spots of gels were identified by using protein database. The t-test showed a significant increase of total proteins in treated group. Cathepsin L- protein (MW 36.7 pH 5.34), 14-3-3 epsilon 2 isoform (MW 28.2 pH 5.36), Cathepsin L1D (MW 36.5 pH 5.8) and Cathepsin L1D (MW 36.6 pH 6.26) were identified in test group. It seems that, these results can be considered for determining the proteins to which the drug is targeted.

**Keywords:** *Fasciola hepatica*, two dimensional electrophoresis, triclabendazole

# PREVALENCE AND ECONOMIC LOSSES OF COMMON ZOONOTIC HELMINTHIC DISEASES IN SLAUGHTERED HERBIVORES IN AMOL AB-ATTOIR, MAZANDARAN PROVINCE, NORTH-ERN IRAN

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Most of the zoonotic helmithic diseases are significantly important in terms of economics as well as public health. This study aimed to investigate the prevalence and economic impacts of common helminthic infections in slaughtered herbivores at abattoir in Amol city. In this descriptive and crosssectional study during 2011 -2014 (first six months) a total of 294099 animals including 200244 sheep, 68641 goats, and 25214 cattle were examined and analyzed at Amol slaughterhouse. A total of 294099 animals were slaughtered at the abattoir, hydatid cyst was observed 12.23 % in liver (35979 cases including 23025 sheep, 10507 goats and 2447 cattle) and 38.86 % in lung (114295 cases including 80726 sheep, 28866 goats and 4703 cattle). Prevalence of Fasciola hepatica and Dicrocoelium dendriticum was recorded in 2.87% cases (8463 cases including 5831 sheep, 1991 goats and 641 cattle) and 2.73 % (8046 cases including 3614 sheep, 4032 goats, and 400 cattle), respectively. Economic losses of helminthic infections in livers and lungs of slaughtered herbivores were estimated 7,768, 560,000 Rials (about 242,764.00 US\$) and 7,906,590,000 Rials (about 247,080.00 US\$), respectively.According to the present investigation, we concluded that the prevalence of common helminthic infections was relatively high in Amol abattoir, which causes great economic losses to the country resulting from the loss of livestock and their products.

Keywords: zoonotic helminths, herbivores, slaughterhouse, economic loss





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## MOLECULAR IDENTIFICATION OF FASCIOLA SPECIES ISOLATED FROM CATTLE AND SHEEP IN SOUTHEASTERN IRAN

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Fascioliasis is an important zoonotic helminth infection in animals and human and is caused by Fasciola hepatica and Fasciola gigantica. Little is known on the etiological agent of fascioliasis in Kerman province. The purpose of the present study was to identify Fasciola species involving in Fasciola infection in different regions of Kerman province in southeastern Iran, using molecular tools. Materials & Methods. Flukes were collected from the livers of 38 naturally infected animal (8 cattle and 30 sheep) in Kerman province. Fresh flukes washed in normal saline and then genomic DNA was extracted from a small portion of the lateral margin of posterior end of each isolate and PCR-Sequencing were performed utilizing mitochondrial cytochrome c oxidase subunit 1 (cox1) gene marker. The sequences were then analysed by NCBI blast and the software Sequence Scanner. Based on the results of cox1 sequence analysis, both F. hepatica and F. gigantica were identified. Out of 38 isolates, 5 flukes (13.1%) were identified as F. gigantica and 33 (86.9%) as F. hepatica. All isolate samples from 30 sheep belongs to Fasciola hepatica and 5 cattle out of 8 were infected with Fasciola gigantica and 3 of them with Fasciola hepatica. Conclusions: The results of the present study provided background information for more efficient diagnosis and control of the diseases in the region.

Keywords: Fasciola gigantica, Fasciola hepatica, molecular identification, cox1, southeastern Iran

# SEROLOGICAL STUDY ON FASCIOLIASIS IN PA-TIENTS REFERRING TO THE SCHOOL OF PUB-LIC HEALTH, TEHRAN UNIVERSITY OF MEDI-CAL SCIENCES, DURING 2008-20014

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Fascioliasis is a zoonotic disease of livestock and human caused by Fasciola species. Here in, the result of serological evaluation of fascioliasis in people referring to the School of Public Health, Tehran University of Medical Sciences during 2008-20014 is presented. Demographic data, symptoms and eosinophil rate were registered for every patient. Using somatic antigen of Fasciola, ELISA was performed and the results were analyzed. Data were analyzed as well. Among 206 applicants, 24.7% were seropositive for fascioliasis, included 45% female and 55% male. Mean age of patients was between 13 to 67 yr. The highest rate of seropositivity was found among 20-30 yr old which coctituted about 39% of all patients. Most of the patients had hypereosinophilia. All patients had history of eating raw vegetables, or drinking unsafe water. Patients were referring from different provinces of Iran. These provinces in descending order included Guilan, Mazandaran, Ardabil, Khuzestan, Lorestan, North Khorasan, Kermanshah, Azerbaijan, Fars, Kordestan, Hamedan and Markazy.During recent years, fascioliasis has been increased in many provinces in Iran. Patients coming from Guilan and Mazandaran provinces were referred early after the onset of their symptoms. Most probably, physicians in this regeions are more alert on fascioliasis than other provinces. Previous wrong diagnosis was more common among patients referring from other provinces than latter provinces.

Keywords: fascioliasis, ELISA, raw vegetable, Iran





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#### PREVALENCE OF LIVER FLUKE INFECTIONS OF SMALL RUMINANTS, IN AHAR ABBATOIR, NORTHWEST OF IRAN

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Fasciola spp. and Dicrocoelium dendriticum are the most known liver parasites of small ruminant in most areas of Iran which cause enormous economic losses in animal husbandry industry of the country. This survey aimed to evaluate the infection rate and its seasonal variation in Ahar city abbatoir, Northwest Iran. In a longitudinal 2-year survey, 8000 small ruminant livers were inspected postmortemly for hepatic trematodes in Ahar abbatoir from early April 2012 to late March 2014. The data were analysed by Anova and T- tests using SPSS 19 software. The results showed the rate of 5.67  $\pm$  1.67 % and 2.31  $\pm$  1.46 % for *Fasciola* spp. and *Dicrocoelium* dendriticum infection respectively. According to results of statistical analysis, fascioliasis was more prevalent than dicrocoeliasis in small ruminants in Ahar whereas no significant statistical differences were observed based on seasons rates in 2-year period of survey. It can be concluded that hepatic trematodiasis is prevalent in Ahar similar to other areas of northwest of Iran and seasonal climatic conditions have no significant impact on the infection rate and related economic losses.

Keywords: fascioliasis, dicrocoeliasis, small ruminants, Ahar, Iran

## PREVALENCE OF FASCIOLIASIS IN RUMINANTS AND THEIR ECONOMIC EFFECTS IN KASHAN, CENTRAL PART OF IRAN

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The aim of the present study was to determine the prevalence and economic losses of Fasciola spp. isolates and costs incurred due to liver condemnation in Kashan from 2012 to 2013. In this cross - sectional study, a total number of 251325 slaughtered sheep, goats and cattle (88939 sheep, 151924 goats and 10462 cattle) were examined via necropsy for fasciolasis in industrial Kashan abattoirs. The average weight of livers was calculated as 1000 g for sheep, 900 g for goats and 5000 g for cattle in the study area. Based on market value, the average price of a kilogram of normal liver in Kashan city during the study period was 8 \$ US. The amount of main nutrients and vitamins elimination in liver contaminated to fascioliasis was determined. Overall, 3.28 % of the livers were infected. For total number of sheep, goats and cattle slaughtered annually in this region, it was estimated that 7505 livers infected and total annual economic losses of fascioliasis of studied animals was 41784 \$ US. (based on market prices in study period). Of this, 23360, 30240 and 15400 \$US was associated with fascioliasis of sheep, goats and cattle, respectively. The results idicated that Fasciola spp. clearly remains prevalent and causes considerable economic losses in cattle, goats and sheep raising areas, including the study area. This study provides baseline data for the future monitoring of this potentially important parasitic infection in the country.

Keywords: economic losses, cattle, sheep, goat, prevalence, fascioliasis





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## RELATIONSHIP BETWEEN PATHOGENIC IN-TESTINAL PARASITIC INFECTION AND CHRONIC AND ACUTE MALABSORBTION

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Malnutrition is one of the most important health problems in developing countries, especially in children. The aim of the present study was to find out the relationship between intestinal parasitic infection and wasting and growth stunting incidence among primary school students living in rural areas of Kashan, Iran. This investigation was conducted with casecontrol design among 195 students from 6 to 11 years old. 65 students with wasting and 65 students with growth stunting were chosen as case groups, also 65 individuals without malnutrition were chosen as control group. The National Center of Health Statistic (NCHS) growth charts was used as normal standard. The nutritional status was measured by Waterlow classification indicators (The ratio of weight to height "wasting" and height to age "stunting" for pathogen intestinal parasites diagnosis). Three serial samples from individuals in case and control groups were collected during different days; and examination of stool samples and cello-tape anal swabs were performed by standard techniques to detect parasite infection. The prevalence of intestinal parasitic infection in students with wasting and students with growth stunting were 16.9% (11 students) and 27.7% (18 students) respectively. 10 individuals with wasting and 14 students with growth stunting have at least one pathogen intestinal parasite. The prevalence of Entamoeba histolytica, Giardia intestinalis, Blastocystis hominis and Enterobius vermicularisin wasting and growth group were 1.5%, 9.2%, 4.6% ,1.5% and 1.5%, 13.8% , 6.2% and 6.2%, respectively. According to data, there was no significant difference between pathogenic intestinal parasitic infections and wasting (P= 0.18) and growth stunting (P=0.08). The results showed that the possibility of causing major detrimental effect on natural status of children by light parasitism is low.

**Keywords:** Intestinal parasitic infections, nutrition disorders, children, primary school

# EFFECT OF OLIVE LEAF, SATUREJA KHUZESTANICA, AND ALLIUM SATIVUM EX-TRACTS ON GIARDIA LAMBLIA CYSTS COM-PARED WITH METRONIDAZOLE IN VITRO

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Giardia lamblia is one of the common causes of worldwide diarrhea in children. Appropriate medicinal treatment for Giardiasis is available but there are some evidences of drug resistance, insufficient efficacy, and unpleasant side effects. In order to reach a more natural drug with suitable efficacy and the lowest side effects, the effects of the hydroalcoholic extracts of olive leaf, Satureja khuzestanica, and Allium sativum on Giardia lamblia cysts were evaluated in vitro. Furthermore, anti Giardial effect of the extracts was compared with metronidazole as the drug of choice. 2 mg and 5 mg of the plants extracts and powder of metronidazole 250 mg pills were added to 1 ml of Giardia lamblia cysts suspension (containing 5000 cyst/ml normal saline), and the percentages of bioavailability of Giardia lamblia cysts were examined at the 2nd and 4th hours after exposure and in 4 and 37 °C temperatures using eosin 0.1 % and a haemocytometer. The data were analyzed by multiway ANOVA test, Tukey's test, and the SPSS software, version 18. The examinations demonstrated that olive leaf extract had the most fatality rate on Giardia lamblia cysts in vitro (37.90  $\% \pm 7.01$  %), followed by the extract of Satureja khuzestanica (32.52 % ± 9.07 %). Metronidazole 250 mg pills had relatively effective fatality rate on Giardia lamblia cysts in vitro (28.75 % ± 10.30 %), whereas Allium sativum (garlic) had the lowest fatality effect on Giardia lamblia cysts in vitro (22.65 %± 10.47 %) With respect to higher fatality effect of olive leaf and Satureja khuzestanica extracts compared with metronidazole in vitro, these plants can be used as suitable candidates to make new anti giardial drugs with low side effects and without drug resistance in the treatment of Giardiasis in children.

Keywords: Olive leaf, Satureja khuzestanica, Allium sativum, metronidazole, Giardia lamblia, in vitro





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Guilan, Iran; 20-22 May 2015 ASIS AND THE PREVALENCE OF GIARDIASIS IN SLAUGH-

# ASSOCIATION BETWEEN GIARDIASIS AND THE LEVEL OF INSULIN IN SERUM

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Giardia lamblia infection was found to be related to micronutrients malabsorption and stunting in children. This gastrointestinal infection is a serious health problem worldwide so that the patients need urgent medical attention. The giardiasis relation with the serum insulin hormone can be highly detrimental to health problem. This relationship was investigated in our study. A total of 28 subjects (6 males and 22 females) with different sex and age groups were chosen from Rudsar clinical laboratory, grouped as Giardia-infected (n=20) and Giardia-free (n=8) samples. The subjects were classified in two age groups as well, the children with below 12 years old and those above that. The serum insulin in these individuals was determined by commercial kits (LIAISON Analyzer). The association between the level of serum insulin and giardiasis was analyzed with independent T-test by the use of SPSS. The healthy subjects with normal glucose tolerance test have shown median basal insulin level of 6.25  $\mu$ lU/ml, the tolerance range was 4-8 µlU/ml. The Giardia-infected group had median basal insulin level of 3.7 µlU/ml, the tolerance range was 2.1 to 4.3 µlU/ml. The difference in the serum insulin level of two groups is highly significant at the 95% confidence level. The significant difference was for both age groups as well. The significant decrease in the mean serum insulin level of the Giardia-infected group can be a demonstration of serious effect on the cells' nutritional status and metabolism. Giardiasis can be a risk factor for decrease of serum insulin level in Rudsar community. The study proposes that there is an urgent need for seeking medical care visits in the presence of possible giardiasis symptoms.

Keywords: giardiasis, infection, serum, insulin

TERED ANIMALS IN URMIA, IRAN Ashkan Saghatforoush<sup>1</sup>

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Giardiasis is among the most common and important gastrointestinal infections. Giardiasis is a diarrheal disease caused by the flagellate protozoa called Giardia Lamblia. Symptoms of the diseases are diarrhea, weight loss, abdominal pain, growth deficiency, malabsorption of sugar, fats and vitamins. This disease is transmitted through contaminated water and food. Giardia is also transmitting via contaminated hands in persons with low hygiene. The parasite usually lives in upper parts of small intestine in the form of trophozoites and transform to resistant cysts while passing in colon. Materials and Methods: In this study, fecal samples were taken randomly from 216 sheep, 93 mature (more than one year) and 123 immature (less than one year). Of these, 45 cases defecate diarrheal feces and 171 cases non-diarrheal feces. Stool samples were examined to detect Giardia cysts and trophozoites by floating on zinc-sulafate solution (33.3%). No Giardia cyst or trophozoite obtained from studied samples. The result indicates that the chance of acquiring giardiasis in studied farms is negative.

Keywords: Giardia, gastrointestinal infection, prevalence, Urmia.





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COMPARISON OF SENSITIVITY OF SUCROSE GRADIENT, WET MOUNT AND FORMALIN-ETHER IN DETECTING GIARDIA LAMBLIA IN STOOL SPECIMENS OF BALB/C MICE

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Giardia lamblia is an intestinal protozoan parasite with various prevalences reported from different parts of Iran. The reasons for differences in prevalence can be diagnostic methods using by different researchers. Therefore, finding reliable, yet simple and inexpensive methods for detection of Giardia cysts is particularly important .The present study was performed to answer the question of "which methods are more sensitive, cheaper and more applicable in diagnostic laboratories". Three methods including sucrose gradient, wet mount and formalin-ether were used on fecal samples of Balb/c mice infected with the Giardia. In this study, 200 stool samples from infected mice (Balb /c) were prepared using abovementioned three methods and studied. Totally, 200 slides for each method were prepared and examined by microscope. It also improved the accuracy check, at each stage the feces of healthy mice were used as negative controls for comparison. Data were analized using SPSS software and then the sensitivity and specificity were calculated for each method. Stool examination of Giardia lamblia infected bulb/C mice showed that from 200 slides for direct method, formalin - ether and sucrose gradient, 101 (50.5%), 166 (83%) and 180 (90%) cases were positive respectively. Therefore, the sucrose method has shown the highest sensitivity 90% compare to the other methods. According to the results of this study, sucrose method is a newer, more sensitive and cost-effective than the two other methods. Therefore, sucrose method is a suitable method, which can used as an alternative of formalin-ether.

**Keywords:** Balb/c mice, diagnostic methods, *Giardia lamblia*, formalin-ether, sucrose gradient

# DETERMINATION OF EFFICACY OF METRONI-DAZOLE AND TINIDAZOLE FOR TREATMENT OF PRIMARY SCHOOL STUDENTS INFECTED WITH GIARDIASIS IN SHIRAZ, 2013-2014

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Giardia intestinalis (also known as duodenalis or lamblia), is one of the most common intestinal parasites of humans worldwide. Infected individuals may be asymptomatic or present dehydration causing diarrhea and abdominal discomfort. Giardiasis can produce chronic diarrhea, lasting for several months, which may result in malabsorption and weight loss, contributing to the increased mortality of individuals who are malnourished or immune deficient in the first 3 years of life. Because of the potential for chronic or intermittent symptoms, treatment is recommended for giardiasis. At least six different classes of drugs are available for giardiasis treatment but it is mainly based on metronidazole. Unfortunately treatment failures in giardiasis occur in up to 20% of cases. Sensitivity and resistance measurement are needed for evaluation of treatment efficacy with available drugs. This descriptive study was carried out from October 2013 to June 2014 among 4th region primary schools of Shiraz for evaluation of metronidazole and tinidazole in infected individual with giardiasis. 91 patients were examined for the therapeutic effects of tinidazole compared to metronidazole. Cases were divided into 2 groups randomly. One group received the standard treatment with tinidazole as a single dose and the other one received metronidazole. The children, resistant to one drug, were prescribed by another drug. They were followed for 10, 20 and 40 days just after the end of therapy for the presence of G. lamblia in their stools. Parasitological cure was documented when there was three times negative stool examination.Of the original 91 patients who were selected, 77 patients completed the study in the follow-up period, 38 patients received tinidazole and 39 cases received metronidazole. After the whole course of treatment, 7.47 (14.9%) in the tinidazole group were found still to have parasites in the stool while 9.40 (22.5%) in the metronidazole group still had the pathogen. The efficacy of tinidazole (85.1%) was more than metronidazole (77.5%).

Keywords: giardiasis, metronidazol, tinidazole





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## MOLECULAR CHARACTERIZATION OF GIARDIA ISOLATED FROM HUMAN USING TPI MARKER IN ISFAHAN

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Giardia duodenalis is one of the most prevalent intestinal parasites of human. It also infects a wide range of mammals. Molecular characterization of G. duodenalis isolates have revealed the existence of eight group (Assemblage A to H) which differ in their host distribution. Two assemblage A and B were commonly reported among humans with different frequency of distribution in different geographical locations. This work was conducted to discriminate genotypes of Giardia duodenalis in human isolates in Isfahan center of Iran by using PCR-RFLP method and tpi gen as DNA markers. This is the first molecular study using triosephosphate isomerase (tpi) markers in human isolates of G.duodenalis in this area. Samples were collected from different health centers of Isfahan from June 2013 to February 2014. From 175 Giardia positive stool samples 67 specimens were selected randomly. Cysts of Giardia positive samples were concentrated by flotation sucrose. Extraction of genomic DNA from trophozoite and cysts was performed using QIAamp Stool Mini kit with a modified protocol. Partial sequences of tpi including 148-bp and 81-bp were amplified for detection of the genotypes A and B using RFLP- PCR protocol respectively. PCR - RFLP assay of 67 isolates with Giardiasis ,showed 40(59.7%) isolates as Genotype A group II, 25(37.31%) samples as Genotype B Group III and mixed genotype of (AII and B) was detected only in two isolates (2.98%). By comparing the frequency of genotype A (81.8%) and genotype B (13.6%), we found that genotype A had six times higher prevalence than genotype B in patients with diarrhea. Conclusions: PCR - RFLP assay targeting Tpi locus is a sensitive tool and discriminates genotypes, sub genotypes and mixed type of G. duodenalis. Results of our study suggest both anthroponotic and zoonotic origins for the infections respectively.

Keywords: Giardia duodenalis, Tpi, polymerase chain reaction, restriction fragment length polymorphism, Isfahan

# THE PREVALENCE OF SOME INTESTINAL PAR-ASITES IN FOOD-HANDLERS OF ASIAN AND AFRICAN COUNTRIES: A META-ANALYSIS

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Parasitic infections are common in many countries, especially in developing countries and tropical areas. People who deal with foods can be a threat to health of communities. This meta-analysis study was undertaken to determine the prevalence of parasitic infections, such as Ascaris lumbricoides, Entamoeba coli, Entamoeba histolytica, and Giardia lamblia, among the food staff in Asian and African countries. We systematically searched Pubmed, Embase, Cochrane library, Proquest, Scopus, and Springer databases. In this meta-analysis, only the cross-sectional studies conducted from 1970 to October 2013 were selected. After searching by the main keywords, 23 papers were found and after applying the inclusion criteria, 11 articles remained. I2 and T2 coefficients were used to find heterogeneity in the studies. Then, random effect model was applied for data analysis by Stata-12 software. The pooled effect size, T2, and I2 for E. coli were 0.041 (CI 95%: 0.009-0.073), 0.004, and 88.9%, respectively. For A. lumbricoides, the pooled effect size was 0.017 (CI 95%: 0.012-0.022), T2 was 0.002, and I2 was 87.2%. These measures were respectively obtained as 0.026 (CI95%: 0.014-0.038), 0.003, and 88.5% for E. histolytica. Ultimately, the pooled effect size, T2, and I2 for G. lamblia were 0.033 (CI95%: 0.022-0.044), 0.004, and 85.7%, respectively. The prevalence rate of some intestinal parasites was relatively low in the food-handlers in Asian and African countries compared to the previous studies. In addition, high heterogeneity was obtained regarding the prevalence rate in these countries.

Keywords: Meta analysis, intestinal parasites, food handler





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# PREVALENCE STUDY OF INTESTINAL PARASIT-IC INFECTIONS AMONG INDIVIDUALS RE-FERRED TO HEALTH CENTERS IN KHORRAM ABAD, LORESTAN PROVINCE, IRAN

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Parasitic infections caused by intestinal helminthic and protozoan parasites are among the most prevalent infections in humans in developing countries, as well as in Iran. Despite widespread progress and reduced morbidity and mortality, still parasitic infection is one of the main sanitary problems in developing countries. In this study 5125 cases that came to take health card and persons referred to central laboratory were evaluated in Khorram Abad city. The result showed the prevalence of infection was 9.6% and protozoic infection was Giardia 4.5%, Entamoeba histolytica 0.2%, Blastocystis hominis 0.17%, Entamoeba coli 0.1% and helminthic infection were Enterobius vermicularis 0.1%, Hymenolepis nana 0.2%, and Strongyloides stercoralis 0.02% .There was significant difference between sex and job (p<0.05). It seems that performing and monitoring of health regulations by health authorities is effective and helpful to control parasitic diseases.

Keywords: intestinal parasitic infections, epidemiology, Khorram Abad

# A SEROLOGICAL STUDY ON CHOLESTEROL, HDL AND LDL CHANGES IN PATIENTS IN-FECTED WITH GIARDIA LAMBLIA IN ARDABIL, IRAN

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Giardia lamblia is a protozoan parasite which contaminates the small intestine of human and many mammals. The outcomes of Giardiasis are diarrhoea (steatorrhea), hypovitaminosis and growth retardation especially in children. This study was conducted in order to determine cholesterol, HDL and LDL levels in the serum of individuals infected with Giardia lamblia. In this descriptive - analytical cross - sectional study the stool samples were prepared from 600 persons referred to Farabi laboratory (Ardabil) during summer and fall 2014 and Giardia infection was investigated using wet mount method. Then, in giardiasis positive cases we provided the blood samples and determined the serum levels of cholesterol, HDL and LDL using autoanalyzer. Finally we analyzed the data by SPSS 19 and independent t-test method. Of 600 stool samples (200 women and 400 men), 25 cases (11 women and 14 men) were positive for Giardiasis. We measured the serum cholesterol, HDL and LDL levels in both case (25 infected cases) and control (25 non-infected persons) groups. The mean of Cholesterol, HDL and LDL were 146.32, 34.88, 94.60 in control group and 175.84, 48.24 and 100.80 in case group respectively. The amount of cholesterol and HDL in case group (giardiasis positive) were higher than control group (cholesterol Sig.= 0.007 and HDL Sig.= 0.008), whereas the amount of LDL not significantly different between the two groups (LDL Sig.= 0.427).

Keywords: cholesterol, HDL, LDL, giardiasis, Ardabil





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# THE PREVALENCE OF GIARDIA LAMBLIA IN WESTERN AREA OF MAZANDARAN PROVINCE, NORTHERN IRAN, 2013-2014

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Introduction & Ojectives: Giardia is a genus of anaerobic flagellated protozoan parasites of the phylum Sarcomastigophora that colonise and reproduce in the small intestines of several vertebrates, causing Giardiasis. The symptoms of Giardia include violent diarrhea, excess gas, stomach or abdominal cramps, upset stomach, and nausea. Current study is aimed to evaluate the status of giardiasis in western area of Mazandaran province. This cross-sectional study was conducted from March 2013 to February 2014 in Kelardasht, Chalous district of Mazandaran province. A total of 1250 stool specimens were collected and examined for intestinal parasites using direct wet mount with saline and formalin ether concentration methods. Statistical analysis was performed using SPSS software version 19. Out of 1250 cases, 119 (9.6%) were positive for Giardia infections. Difference in prevalence of giardiasis was not statistically significant between males and females. On the other hand, difference in the prevalence of giardiasis was statistically significant between age groups. The results on current study showed fairly low amount of giardiasis in our study population. This may be due to high quality of health services and education of people.

Keywords: Giardia lamblia, prevalence, giardiasis, Iran

IN VITRO CULTURE OF GIARDIA DUODENALIS FROM FECAL MATERIALS

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Giardiasis is one of the common intestinal infections occurring worldwide. Understanding the biological characteristics of Giardia duodenalis is important, especially in pathology causes by the pathogenic strain. The main objective of this study was to effectively culture G. duodenalis from human fecal materials. Human Fecal samples of patients were obtained from Shiraz University of Medical Sciences. Purification of G. duodenalis cysts from stool was done using two step sucrose gradients with the specific gravity of 1.5 M and 0.75 M on positive stool samples. Excystation was performed by incubating the purified samples in the solution prepared by mixing 1 g of pepsin, 0.1 g of L-cysteine hydrochloride, and 0.1 g of ascorbic acid in 100 ml of normal saline (pH 2) for 1 hour at 37°C. The axenic culture was initiated in a modified TYI-S-33 culture medium (Keister, 1983) inside culture tubes filled up to 80% of the total volume. TYI-S-33 culture medium for axenic cultivation of G. duodenalis was prepared by mixing 2.0 ml of vitamin mixture number 18 and 10 ml of heat-inactivated adult bovine serum; this as then was added to each of the 88 ml of TYI broth solution. Antibiotic and antifungal were added to the final working solution of TYI-S-33. The culture tubes were then incubated at 35.5°C horizontally and inspected daily with an inverted microscope until a monolayer of G. duodenalis was formed, usually between 48 to 72 h. The trophozoites were passaged at every 72 h and 96 h intervals, twice weekly. Successful axenization could be achieved in modified TYI-S-33 medium (Keister, 1983). In this study, continuous axenic culture of G. duodenalis isolates from patients was established.Modified TYI-S-33 was the medium used for the cultivation of G. duodenalis that successfully maintained this fastidious parasite. This was the most suitable medium for mass cultivation of active and viable G. duodenalis trophozoites for further analysis. Activating the cysts and excitation process was a first step for cultivation. Contamination was another challenging that needs effective and suitable antibiotics. Inspecting visually culture tubes with an inverted microscope daily was necessary. The medium should be decanted and replaced with fresh medium and antibiotics. Establishment of the axenic culture of G. duodenalis is the most important part of the supply of the parasite to understand better on the population genetics, taxonomic situation and epidemiological importance of Giardia in Iran. In vitro cultivation isolated G. duodenalis is able to provide enough number of parasites needed for taking effective measures through molecular biology research, vaccine production, drug resistance, control and treatment strategies of human giardiasis.

Keywords: Giardia duodenalis, axenic culture, TYI-S-33, Iran





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GIARDIA INFECTION IN STRAY DOGS FROM GUILAN, MAZANDARAN AND GHAZVIN PROV-INCES, IRAN

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Giardia duodenalis is one of the major diarrhoea agents in human and animals throughout the world. Assemblage A and B of this protozoa have been detected in humans and a wide range of animals particularly dogs. Therefore, dogs could act as transmitter of this pathogen to human. The aim of this study is to detect Giardia infection in stray dogs in north of Iran. 85 stray dogs were collected from Guilan, Mazandaran and Ghazvin provinces during the period September to March 2012. They euthanized and necropsied for other purpose in Tehran Small Animal Research and Teaching Hospital. Duodenal mucosa of each dog was scraped and smear prepared. The stool smears were prepared too. Briefly, a drop of fecal suspension was placed on a glass slide and spread to form a thin smear. Microscopic diagnosis of Giardia was performed with a Giemsa stain with slight modification. Slides were fixed in hot methanol for 5 min and then flooded with Giemsa for 45 minutes. After washing, slides were examined under 100X objectives. 4 out of 85 duodenal mucosa smears were positive for Giardia with a Giemsa stain. According to this method, the prevalence rate of giardiasis in stray dogs was 4.7%. None of the stool smears showed Giardia. The staining of stool smears couldn't be a reliable method to detect Giardia, due to intermittent shedding of Giardia. The smears prepared from duodenal mucosa is a valuable method but not easy to do. The prevalence of the Giardia in dogs in this study isn't high, but the zoonotic risk for human is important and must be considered.

Keywords: Giardia, stray dogs, Iran.

# DNA EXTRACTION FROM GIARDIA INTESTI-NALIS: ASSESSING FOUR APPROACHES TO IM-PROVE REAL TIME PCR RESULTS

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Giardia intestinalis, one of the most prevalent intestinal parasites in the world, causes gastroenteritis and diarrhea. DNA extraction for species identification and molecular diagnosis of parasites is important in molecular surveys. The amount of DNA concentration and purity is very important to perform molecular methods. Because of the high solidity of the Giardia cysts wall, the extraction of its DNA faces with difficulty. The purpose of this study was to evaluate the effect of 4 methods to make better disruption and increased DNA yield. In this study 70 cysts of infected fecal samples was concentrated by flotation sucrose and then 4 methods including: 1crushed cover glass, boil, freeze-thaw, Gene All kit, 2-glass bead, freeze-thaw, Gene All kit, 3- 2Me, freeze-thaw, Gene All kit, and 4-Crushed cover glass, lysis buffer, boil were used for DNAextraction and in the next step Real time PCR reaction performed on tpi target gene. The results of present study indicated that the first method had the best effect on DNA extraction with 100 percent and in the next places second method with 56.41%. Third method with 20 percent and fourth method with 11.11 percent were identified.Consequently, the pretreatment of cysts with crushed cover glass, boil and freeze-thaw cycles followed by extraction of DNA with the Gene All kit was the more effective protocol.

Keywords: Giardia intestinalis, Real Time PCR, DNA extraction





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#### Guilan, Iran; 20-22 May 2015 GIARDIA LAMBLIA IMMUNE DIAGNOSING BY DOT BLOT TECHNIQUE FECTIO

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Giardia lamblia is a worldwide parasitic infection. This infection is more common in rural and less developed area. Stool examination is a routine method for diagnosis of giardiasis. Besides its advantages, this technique is not able to detect low intensity infections. So the aim of this work was to find a simple and specific method for detection of Giardia infection which can be used in rural area. Giardia lamblia infected stool samples were collected from different medical labs. Giardia cysts were purified using sucrose method. The purified cysts were then washed sonicated and injected to rabbit with frond adjuvant. Antibody production against Giardia antigen in rabbit was confirmed by detection of anti-Giardia antibody in rabbit serum. Then dot blot technique for detection of Giardia was set up using Giardia antigen. According to result of this work, detection of Giardia infection in stool samples is under investigation.

Keywords: dot blot, Giardia, diagnosis

PREVALANCE OF INTESTINAL PARASITIC IN-FECTIONS IN JIROFT, KERMAN PROVINCE, IRAN

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Intestinal parasitic infections have a worldwide distribution. High prevalence is found in people with low socio-economic status and poor environmental conditions. Since no study has ever been conducted on this issue we aimed to evaluate the prevalence of these infections in Jiroft. A total of 1060 individuals from rural and urban areas of Jiroft were selected randomly during 2013-2014. Fresh stool samples were collected from all individuals and examined by formalin-ether concentration and agar plate culture. Direct examination was performed on watery samples. All the samples were confirmed for parasitic infections in the Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences. Out of 1060 individuals, 563 people (53.1%) and 497 people (46.9%) were from rural and urban areas, respectively. In general, 297 individuals (28 %) were infected with intestinal parasites. The prevalence of infection for protozoan and helminthic infections were 27.4% and 1.8%, respectively. The most prevalence pathogenic protozoan was Giardia lamblia (7.8%), and that of helminth was Hymenolepis nana (1.1%). Intestinal protozoan parasites are more prevalent than helminth parasites. Water supply and personal hygiene and important factors in the distribution of the prevalent parasites in the study area should be considered.

Keywords: prevalence, intestinal parasites, Jiroft, Iran





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INFECTION OF GIARDIA LAMBLIA AND BLAS-TOCYSTIS HOMINIS IN INDIVIDUALS WITH

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ABDOMINAL SYMPTOMS

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Some factors such as, unsafe water supply, poor living condition, low socio-economic status, poor environmental sanitation are the important cause of burden of the parasitic diseases in the rural area in developing countries. In this study we investigated the correlations between the personal data and the presence of abdominal symptoms with the frequency of Giardia lamblia and Blstocystis hominis in population living in the rural communities. The study was carried out in October 2011 in the four villages of Kabutare Ahang in Hamadan province. Stool samples were collected from adults and children aged 3 to 63 years (86 males and 49 females) referred to our field laboratory by local physician. All of individuals had abdominal symptoms. The stool samples were examined by formalin-ether concentration and direct wet mount procedures. Significant association was determined using Chi squared and Fisher's exact tests. From the 135 surveyed population, 45 (33.3%) were positive for Giardia lamblia and Blastocystis hominis parasites and 7 (5.2%) were infected with Giardia, 27 (20%) with Blastocystis and 11 (8.14%) were infected with both protozoa. The infections were more prevalent in children below 12 years age group and male sex. The results revealed a correlation between these protozoa infections and living conditions in our study group, but did not show any significant difference between abdominal pain and parasitic infection when compared with other causes of abdominal symptoms (P>0.05).

Keywords: Giardia, Blastocystis, abdominal symptoms, Hamadan PREVALENCE OF GIARDIA INFECTION IN FOOD SUPPLIERS /DISTRIBUTORS REFERRED TO CENTRAL LABORATORY OF KAHNOUJ, IRAN IN 2014

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Due to the importance of intestinal parasitic infections in personal and public health, role of food suppliers and distributors in contamination of foodstuff and transmission of infectious diseases and considering the symptomless nature of the infection in majority of them, early detection and treatment of these infections are critical. The aim of this study was to determine the prevalence of Giardia infection among the food supplier and distributors referred to central laboratory of Kahnouj, in 2014. In this cross sectional study, all present data of the food suppliers and distributors who referred to central laboratory of Kahnouj were collected. These data were analyzed using SPSS version 17. The diagnostic method was the preparation of direct smear from the stool samples in three consequent days or with a day interval. Out of 1990 results, 53 (4.4%) had a greater incidence of Giardia and 80% of them were male. Average age of participants was 26 years. About 70% of infected people were asymptomatic. Seasonal prevalence showed more incidences in July, August and October. According to the results, Giardia infection has more incidence compared to other parasites. Therefore, to prevent this infection, intermittent checkup should be undertaken on individuals who are dealing with food supplies

Keywords: Giardia, Kahnooj, prevalence





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# PREVALENCE OF HYDATID CYST IN SLAUGH-TERED LIVESTOCK IN JAHROM DURING 2012 -2014

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Hydatid cyst disease caused by the larval stage of *Echinococcus* granulosus is still one of the most important factors of economic and health problems in different parts of the world. The purpose of this study was to determine the prevalence of hydatid cyst in livestock slaughtered in an abattoir of Jahrom. Material & method: In this descriptive cross-sectional study, the slaughtered livestock was evaluated from September 2012 to September 2014. Type of animals, the numbers of slaughtered animals, and infected tissues was recorded. The data were analyzed by descriptive statistics using SPSS software. Result: The total numbers of slaughtered livestock were 78000, of which 71.5%, 23.7%, and 4.8% were goats, sheep, and cattle respectively. The percent of hepatic and pulmonary hydatid cyst was 3.6 (2861 cases) and 4.5 (3561 cases) respectively. The highest prevalence of hydatid cyst was related to cattle with 16.8 % in the liver and 23.12 % in the lung in the 2012. The rate of infections in the first 6 months of the year was higher than the second 6 months. The rate of infection was higher in cattle than other livestock. Protection of pastoral zone and elimination of stray dogs has critical role in reducing the risk of livestock and human infections.

Keywords: hydatid cyst, prevalence, slaughtered livestock, Jahrom

# PREVALENCE OF HYDATID CYST IN SLAUGH-TERED LIVESTOCK IN JAHROM DURING 2012 -2014

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Hydatid cyst disease, caused by the larval stage of Echinococcus granulosus, is still one of the most important economic and health problems in different parts of the world. The purpose of this study was to determine the prevalence of hydatid cyst in livestock slaughtered in a slaughterhouse of Jahrom. Material & method: In this descriptive cross-sectional study, the livestock slaughtered was evaluated from September 2012 to September 2014. Type of animals, the numbers of slaughtered livestock, and infected tissues was recorded. The data were analyzed by descriptive statistics using SPSS software. The total numbers of slaughtered livestock were 78000 of which 71.5%, 23.7%, and 4.8% were goats, sheep, and cattle respectively. The percent of hepatic and pulmonary hydatid cyst was 3.6 (2861 cases) and 4.5 (3561 cases) respectively. The highest prevalence of hydatid cyst was related to cattle with 16.8 % in the liver and 23.12 % in the lung in 2012. The rate of infectious in the first 6 months of the year was higher than the second 6 months. The rate of infection was higher in cattle than other livestok. Thus, control of dogs feeding and pastural zone in addition to elimination of stray dogs are needed.

Keywords: hydatid cyst, prevalence, livestock, *Echinococcus* granulosus, Jahrom





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# EVALUATION THE KNOWLEDGE LEVELS RE-GARDING HYDATID CYST AMONG BUTCHERS IN KARAMAN PROVINCE OF TURKEY

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Hydatid cyst is a globally zoonotic disease, which is an important public health problem and has economical impact. This descriptive research aims to determine the knowledge levels of butchers regarding hydatid cyst in Karaman province of Turkey. On July 2014 a questionnaire consisting of 10 items was prepared for butchers. Questionnaire form including basic information and public health subjects for hydatid cyst was applied to 25 butchers with face-to-face meeting. 95% of the butchers stated that they have some information about hydatid cyst and it was emphasized that they encountered the disease while slaughtering. 50% of butchers stated that they put in the ground the infected organ while 45% cast away and 5% give them to dogs. The information of the animal's cysts can be infective for humans, dogs are responsible for transmitting the disease, not washing the vegetables that raw consumed play role in transmission of the disease and dogs are reservoir for some other disease was known correctly with the percentages of 80%, 60%, 50% and 90%, respectively. However 65% of butchers point out that they think "Vets don't examine the internal organs while slaughtering carefully". 80% of the participants say that they don't keep a dog and 95% of them reported they didn't participate any informative course about hydatid cyst.It was detected that the butchers have insufficient information for the disease and it is recommended that information studies should be performed for butchers across the Karaman province and in Turkey urgently.

Keywords: hydatid cyst, butcher, knowledge, Turkey

# MOLECULAR CHARACTERIZATION OF ECHI-NOCOCCUS GRANULOSUS STRAIN IN STRAY DOGS IN MASHHAD

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Cystic echinococcosis (CE), caused by *Echinococcus granulosus* is one of the most important zoonotic diseases and is prevalent in different parts of Iran. With the aim of genotyping *Echinococcus granulosus* found in small intestine of stray dogs, 20 adult worms of *E. granulosus* were collected from 100 dogs from Mashhad in northeast of Iran.Adult worms were genetically characterized using PCR-RFLP analysis of ITS1 gene with the restriction endonuclease Bsh1236I. The present results have shown that all of 20 *E. granulosus* worms were determined as the G1-G3 strain. The nucleotide sequence of this genotype showed 99% homology with the G1 sequence.The presence of G1 genotype (sheep strain) of *E. granulosus* sensu stricto as dominant genotype in stray dogs in Mashhad in northeast of Iran is emphasized.

Keywords: Echinococcus granulosus, Mashhad, PCR- RFLP





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 MOLECULAR CHARACTERIZATION OF ECHI FERTILI

 NOCOCCUS ISOLATES IN CATTLE SLAUGH FERTILI

 TERED IN MASHHAD AND AHVAZ
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Cystic hydatid disease (CHD), caused by *Echinococcus granulosus* is one of the most important zoonotic diseases, and is prevalent in different parts of Iran. The aim of the present study was to genotyping the hydatid cysts obtained from cattle in Mashhad and Ahvaz provinces. Fifty hydatid cysts from slaughtered cattle in Mashhad and 20 from cattle in Ahvaz were studied. Germinal layer collected from these cysts were genetically characterized using PCR-RFLP analysis of ITS1 gene with the restriction endonuclease Bsh1236I. The present results have shown that all of 70 hydatid cysts were determined as the G1-G3 strain. The nucleotide sequence of this genotype showed 99% homology with the G1 sequence. The presence of G1 genotype (sheep strain) of *E. granulosus* sensu stricto as dominant genotype in cattle in Mashhad and Ahvaz area is emphasized.

Keywords: Hydatid cyst, cattle, Mashhad, Ahvaz, PCR-RFLP

FERTILITY RATE OF HYDATID CYSTS IN CAT-TLE WITH INCREASING AGE

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Hydatid disease or echinococcal disease, is a parasitic disease that affects both humans and other mammals, such as sheep, dogs, rodents and horses. There are three different forms of echinococcosis found in humans, each of which is caused by the larval stages of different species of the tapeworm of Genus Echinococcus. Fertility of protoscolices of hydatid cysts in infected organs of intermediate hosts has important role in the epidemiology of Echinococcus and finally hydatidosis. Considering the importance of the fertility of hydatid cysts, a study on beef cattle was proposed. Material & Method: During the one year by referring to the Slaughterhouse, sampling has been carried out of the 100 livers cyst. After collecting the organs of infected cysts, according to age group, first a sterile cyst fluid was taken out of the cyst, each sample separately collected in a glass container to be fertile, and sterile cysts were identified in this phase. The results show that fertility rates in young animals (under 5 years) were less than 50%. However, at the age over 5 years, the fertility rate reached to 75%. With increasing age, the fertility rate of cysts increased. This is probably because in the young animals, cysts are still young and have no chance of reproduction.

Keywords: Fertility rate, hydatid cysts, cattle, age





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# THE EFFECT OF ALBENDAZOLE AND MEBEN-DAZOLE ON THE VIABILITY OF HYDATID CYST PROTOSCOLICES, IN VITRO

# Jafar Adnani Sadati<sup>1</sup>, Ali Farahnak<sup>2</sup>, Mohammad Bagher Molaei Rad<sup>3</sup>, Abolfazl Golestani<sup>4</sup>, Mohammad Reza Eshraghian<sup>5</sup>, Iraj Mobedi<sup>6</sup>, Mehdi Mohebali<sup>7</sup>

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Hydatidosis is caused by the larval stage of the Echinococcus granulosus in humans and domestic animals. Benzimidazoles are used for treatment of this disease in human. The aim of this study was to investigate the effect of albendazole and mebendazole on the viability of protoscolices in vitro by the same condition in two different cultures media. Materials & Method: After collection of hydatid cyst livers from sheep slaughtered in the slaughterhouse, the studies curried on the fertile cysts. The protoscolices were evacuated, washed and their viability was examined. The certain volumes of protoscolices were poured into Falcon tubes containing culture media (RPMI-1640/PBS, pH 7.2) and albendazole and mebendazole solutions were added with final concentration 1 µg/ml. The protoscolices were recollected after specific time interval and their viabilities were examined by staining with Eosin under an optical microscope. The results showed that albendazole is more effective in removing protoscolices. In albendazole group, the amount of viability of protoscolices came to zero after 35 days, while the viability of protoscolices in the mebendazole group lasted about 42 days (p < 0.05). The percentage of viability of protoscolices in two media (PBS and RPMI 1640) at the same time did not show significant statistical differences (P<0/05). According to the results of this research, albendazole can be used as a suitable drug in the elimination of hydatid cyst protoscolices and for prevention of the relapse of this disease. PBS can be used as a simple and affordable medium for protoscolices cultivation in vitro.

Keywords: hydatid cyst, protoscolex, albendazole, mebandazole, culture media

# THE EFFECTS OF CONTINUOUS AND REPETI-TIVE MICROWAVE RADIATION ON PROTO-SCOLICES OF HYDATID CYST

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Hydatidosis is a zoonotic disease causd by parasitic worms of the Genus Echinococcus. The main method of treatment is surgery, but several studies are being done to reduce the risk of surgery. The microwaves cause microbial destruction by thermal and nonthermal effects. The aim of our study was to investigate the effect of continuous and repetitive microwaves radiation on protoscolices of hydatid cyst. Liver hydatid cysts of naturally infected sheep were obtained from abattoir. Protoscolices were aspirated from cyst and were transferred into a dark container and stored at 4°C for further use. Experiments were conducted in two separate groups. These groups received continuous and repetitive irradiations. The results showed that microwave has lethal effects on protoscolices. When protoscolices was constantly exposed to radiation, increase of mortality rate was directly related to exposure time and temperature increase. When repetitive irradiation was used, temperature change had no significant effect. However, longer exposure times were needed to kill all protoscolices.Microwaves can be used as a supplementary measure to both treat and prevent hydatid cysts. This method is noninvasive, and therefore promises to minimize the risk of cyst rupture. However, extensive application of the method requires additional research in this issue.

Keywords: microwave, hydatid cyst, protoscolices





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# SCOLICIDAL EFFECTS OF CUMINUM CYMINUM L. ESSENTIAL OIL AGAINST HYDATID CYST PROTOSCOLICES

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Cuminum cyminum L. (Apiaceae family) commonly named "Cumin seed" is an aromatic plant with various pharmacological activities such as antioxidant, anticancer, anticoagulant, and antimicrobial mentioned in traditional Iranian medicine and modern phytotherapy. The present study was designed to evaluate the scolicidal effects of C. cyminum essential oil on the protoscolices of Echinococcus granulosus on in vitro model. To assess the scolicidal effects, protoscolices were aseptically aspirated from the naturally infected livers of sheep and goats. Various concentrations of essential oil were used for 5-30 minutes. Eosin exclusion test was used to determine the viability of protoscolices. Findings showed that C. cyminum essential oil at the concentrations of 50 and 100  $\mu$ l/mL killed 100% protoscolices after 5 and 10 minutes of exposure, respectively. In contrast, the mortality rate of protoscolices in the control group (normal saline) was 4.3% after 60 minutes exposure. The results obtained in this investigation for the first time demonstrated that C. cyminum essential oil might be a natural source for the production of new scolicidal agents for use against hydatid cyst in surgery.

**Keywords:** hydatidosis, *Echinococcus granulosus*, hydatid cyst, *Cuminum cyminum* 

# MORPHOLOGICAL AND MOLECULAR STUDIES (ITS1) OF HYDATID CYSTS IN SLAUGHTERED SHEEP IN BOJNORD AREA

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Cystic echinococcosis (CE) is a global zoonotic infection which is economically important and constitutes a threat to public health in many countries. Echinococcus granulosus have ten strains from G1 to G10. Each strain is related to a special intermediate host. The morphology, epidemiology, treatment and control of these strains are different. There are various molecular methods to differentiate between Echinococcus strains. Morphological and molecular methods provide better information about identification of each strain. The aim of this study was to identify Echinococcus granulosus strain of hydrated cysts in slaughtered sheep in Bojnord area. In the present study, the infected liver and lung with hydatid cysts were collected and transferred to laboratory. The hydatid cyst liquid was extracted and morphological characters of rostellar hook protosclocies were measured using ocular micrometer. The total length of blade of large hooks, blade length of small hooks, and number of hooks per protoscolex were, 24.61±0.4, 12.47±0.5, 19.66±0.5, 9.1±0.4 and 33.4±1.6, respectively. In molecular section of the study, DNA of each sample was extracted with MBST Kit and reaction was carried by PCR using special primers (EgF, EgR) which amplified fragment of ITS1 gen. The PCR product was digested with Bsh1236I enzyme. Based on pattern of PCR-RFLP results (four band forming), G1, G2 and G3 strain of Echinococcus granulosus were obtained. Differentiation of three strains was done using sequencing analysis and G1 strain was diagnosed. The agreement between the molecular results with morphometric characters of rostellar hook confirmed the presence of G1 strain of *Echinococcus* in the slaughtered sheep of Bojnord area.

Keywords: strain, hydatid cyst, PCR, sheep





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# COMMON ANTIGENS BETWEEN HYDATID CYST AND CANCERS

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Different research groups reported a negative correlation between cancers and parasitic infections. As an example the prevalence of hydatid cyst among patients with cancer was significantly lower than its prevalence among normal population. Tn antigens exist both in cancer and hydatid cyst. This common antigen may involve in effect of parasite on cancer growth. So in this work common antigens between hydatidcyst and cancers have been investigated. Different hydatid cyst antigens including hydatid flouid, laminated & germinal layer antigens and excretory secretory antigens of protoscolices were run in SDS PAGE and transferred to NCP paper. In western immunoblotting those antigens were probed with sera of patients with different cancer and also sera of non cancer patients. Also cross-reaction among excretory secretory products of cancer cells and antisera raised against different hydatid cyst antigen was investigated. In western immunoblotting antisera raised against laminated & germinal layers of hydatidcyst reacted with excretory secretory products of cancer cells. In addition, a reaction was detected between hydatid cyst antigens and sera of patients with some cancers. Coclusion: Results of this work emphasises existence of common antigens between hydatid cyst and cancers. More investigation about these common antigens is recommended.

Keywords: hydatid cyst, cancer, common antigen

EFFECTIVENESS OF METHANOLIC EXTRACT OF ZATARIA MULTIFLORA ON HYDATID CYST IN LABORATORY MICE

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The phenolic compounds of Zataria multiflora extract were identified by HPLC analysis. Gallic acid, catechin, caffeic acid, and quercetin were found to be the major phenolic compounds. Materials and Methods: Eighty healthy laboratory Balb/c mice were infected intraperitoneally by injection of 1500 viable protoscolices and were divided into prevention (40 mice) and therapeutic (40 mice) groups. To prove the preventive effect of Z. multiflora extract on development of hydatid cyst, the 40 infected animals were allocated into three treatment groups includ-ing Z. multiflora (4 g/l in drinking water for 8 months), albendazole (150 mg/kg BW/day for10 days) and untreated (control) group. To estimate the therapeutic effect of Z. multiflora extract on the hydatid cyst, after 8 months of infection, the infected mice were allocated into three experimental treatment groups including Z. multiflora (8 g/l in drinking water for 30 days), albendazole (300 mg/kg BW/day for 20 days) and untreated (control) group. At the end of the treatment period, all mice were euthanized and necropsied, the hydatid cysts were carefully removed, weighed and their size were recorded. Result: Weight and size of the hydatid cysts significantly decreased (p < 0.05) upon the treatment with Z. multiflora extract in both prevention and therapeutic groups. The germinal layer of the hydatid cysts recovered from the treated mice, either from the prevention or therapeutic group, were completely damaged at ultrastructural level by scanning electron microscopy.

**Keywords:** preventive, therapeutic, methanolic extract, *Zatar-ia multiflora*, hydatid cyst, in vivo





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EVALUATING THE GENE EXPRESSION LEVEL OF IL-12 IN THE FIBROUS LAYER OF HEPATIC HYDATID CYSTS ISOLATED FROM SHEEP AND GOAT

#### <u>Roghiyeh Faridnia</u><sup>1</sup>, Sepideh Tolouei<sup>2</sup>, Hosein Khanahmad-Shahreza<sup>3</sup>, Hamed Kalani<sup>2</sup>, Hosein Yousefi-Darani<sup>2</sup>

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Hydatidosis is a zoonotic disease with worldwide distribution. This disease is able to induce strong immune responses in the infected host. This study aimed at evaluating the gene expression level of IL-12 in the fibrous layer of hepatic hydatid cysts in bovine and ovine samples. For each one of the bovine or ovine groups, 12 samples was considered (6= control and 6= experimental). The experimental samples were collected from the fibrous layer of the bovine and ovine hepatic hydatid cysts and the control ones from the normal liver tissues. Thus, the overal number of samples was 24. The total RNA was extracted from samples and then converted to cDNA. Subsequently, the gene expression level of IL-12 in both test and control groups of the bovine and ovine samples was measured using quantitative reverse transcription (Q-RT)-PCR procedure. The results showed that IL-12 gene expression level in the fibrous layer of ovine hydatid cyst samples (experimental group) was 0.74-fold that of the ovine normal liver tissue (control group). Moreover, IL-12 gene expression level in the bovine normal liver tissue (control group) was 2.96-fold that of the fibrous layer of the bovine hydatid cyst samples (experimental group). In addition, there was a significant deference between the bovine and ovine samples (p < 0.01). It can be concluded that the bovine hydatid cyst is probably able to induce apoptosis in leukocytes accumulated in the fibrous layer. Therefore, there is a possibility of using compounds of the bovine hydatid cyst in the induction of apoptosis in cancer cells.

Keywords: cattle, sheep, hydatid cyst, IL-12, gene expression

# PREVENTIVE AND THERAPEUTIC EFFECTS OF THE AROMATIC WATER OF ZATARIA MULTI-FLORA ON HYDATID CYSTS: AN IN VIVO STUDY

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Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) were employed to determine the chemical composition of the essential oil (EO) from aromatic water (AW) of Zataria multiflora. Thymol (66.9%), carvacrol (15.2%), and carvone (7.3%) were found to be the major EO constituents. Materials and Methodes: Eighty laboratory Balb/c mice were infected intraperitoneally by injection of 1,500 viable protoscolices and were divided into prevention (40 mice) and therapeutic (40 mice) groups. To prove the preventive effect of the Z. multiflora AW on development of hydatid cysts, the 40 infected mice were allocated into three treatment groups, including the albendazole group (10 mice that received 150 mg/kg body weight/day for 10 days), the Z. multiflora AWgroup (15 mice that received 20 ml/liter in drinking water for 8 months), and a control group (15 mice that received no treatment). To estimate the therapeutic effect of Z. multiflora AW on the hydatid cyst, after 8 months of infection, the 15 remaining mice were allocated into three experimental treatment groups of five animals each, including the albendazole group (300 mg/ kg/day for 20 days), Z. multiflora AW group (40 ml/liter in drinking water for 30 days), and control group (no treatment). All mice were then euthanized, and the sizes and weights of the cysts as well as their ultrastructural changes were investigated. The weights and sizes of the hydatid cysts significantly decreased upon treatment with the Z. multiflora AW in both the preventive and therapeutic groups (P<0.05). The results of scanning electron microscopy also showed considerable damage in the germinal layer of the hydatid cysts recovered from the treated animals.

Keywords: aromatic water, Zataria multiflora, hydatid cyst, mice, in vivo





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ECHINOCOCCUS GRANULOSUS GENOTYPES IN IRAN

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Hydatidosis, caused by Echinococcus granulosus is one of the most important zoonotic diseases worldwide. Hydatidosis is endemic in Iran and responsible for approximately 1% of admission to surgical wards. There are extensive genetic variations within E. granulosus and 10 different genotypes (G1-G10) within this parasite have been reported. Identification of strains is important for improvement of control and prevention of the disease. No new review article presented the situation of Echinococcus granulosus genotypes in Iran in the recent years; therefore in this paper we reviewed the different studies regarding Echinococcus granulosus genotypes in Iran. In this review article, we used electronic resources such as Google Scholar and Pubmed. Echinococcus granulosus and Genotype as keywords from all articles have been used in different years. In this article the situation of hydatid cyst genotypes have been reviewed. Based on studies performed in different regions of Iran, presence of G1, G2, G3 and G6 genotypes were reported. Molecular studies demonstrated the presence of several genotypes including sheep strain (G1) in sheep and human, Tasmanian strain (G2) in sheep and humans, the pig strain (G7) in pigs, and the camel strain (G6) in humans. In Nepal, hydatid cyst is a significant public health and environmental problem, three strains including sheep (G1), cattle (G5) and camel (G6) have been reported from buffalo, sheep, goat and human hosts. In comparison of hydatid cyst genotypes in Iran with other countries, 4 strains have been reported from Iran whereas in china, Kenya, Nepal and Argentina genotypes 2, 2, 3 and 4 have been reported, respectively. Therefore, it is obvious that Iran is a country containing more variation of these parasite genotypes.It should be emphasized that hydatid cyst exists with genotype variation in Iran and the majority of E. granulosus infected domestic animals can potentially act as reservoirs of human infection. Therefore, this diversity should be considered in prevention programs.

Keywords: Echinococcus granulosus, hydatid cyst, genotype, Iran

EPIDEMIOLOGICAL SURVEY OF HYDATIDOSIS IN EAST AZARBAIJAN PROVINCE, IRAN (2011-2013)

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Hydatidosis is one of the most important zoonotic diseases affecting almost all vital organs. Echinococcosis is widespread and it is an endemic infection in Iran. The aim of this survey was to study the hydatidosis among operated patients in East Azarbaijan province. The data was extracted from the files of operated patients who were admitted to general surgery ward. Patients were studied for age, sex, job, place of residence, location of cyst, signs and symptoms of disease. From 70 cases of hydatidosis, 64% were female and 36% were male. Most of the patients were housewives (55%). The highest rate of infection was seen in 20-59 age groups (78%). The highest rate of infections was observed in liver with 70%, lung with 14% and in both liver and lung with 9%. Control of infection in this area and similar regions should be considered by health authorities.

Keywords: hydatic cyst, East Azarbaijan, epidemiology





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EVALUATION OF HYDATID CYST RATE IN LIVESTOCK SLAUGHTERED IN CHALUS AND MARZANABAD, NORTHERN IRAN IN 2012

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Hydatidosis is a zoonotic disease causes important health problems and economic losses worldwide.Hydatid cyst disease is caused by parasitic worms in baby steps called "Echinococcus granulosus". The disease transmits to humans through close contact with infected dogs or eating unwashed vegetables and fruits contaminated with dog fecal materials. Hydatidosis is more prevalent in areas with traditional herding and villagers at the higher risk of the infection. Materials nd Methods: The aim of the present study was to evaluate the prevalence of hydatid cyst in sheep, goats and cattle in Chalus and Marzanabad slaughterhouses to estimate the economic losses to the livestock industry. Of 1997 livestock slaughtered in Marzanabad, 64 (3.2%) were infected of which 28 (43.8) were sheep, 28 (43.8%) goats and 8 (12.4%) cattle. From 10,563 slaughtered livestock in Chalus district 139 (1.3%) were infected with hydatid cyst; 51 (36.7%) sheep, 28 (20.1%) goats and 60 (43.2%) cattle.Based on the results, the rate of infection was high in studied area which cause in significant economic loss.

Keywords: hydatidosis, *Echinococcus granulosus*, Chalus, Marzanabad EFFECT OF POLYSPECIFIC ANTISERA AGAINST HYDATID CYST ANTIGENS ON MELANOMA TOUMOR GROWTH IN MOUSE MODEL

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Hydatid cyst is the larval stage of Echinococcus granulosus, a parasite responsible for hydatidosis in human and livestock. The effect of different antigens of this parasite in preventing the growth of tumor cells has been demonstrated in various studies. Therefore, in this study the effect of the injection of antisera against antigens of hydatid cyst on Melanoma cancer growth in animal models has been investigated. In this experimental study, C57/black mice were subjected to injection with melanoma cells and then antisera against different antigens of hydatid cyst, prepared in rabbits, were injected to these mice. Mean tumor area in case groups were measured and compared with mean tumor area in control groups. Then, the data were analysed using SPSS software and oneway anova test. The mean tumor area in mice that received antisera against cyst fluid, protosculex crude antigen, excretory-secretory antigens of protosculex and cuticular layer were not significantly different from those of control mice. The results of this study showed that injection of antisera against antigens of hydatid cyst in mice recieved melanoma cells had no siginificant effect on tumor growth. So it is recommended that effect of transfer of immune cells to be investigated in future studies.

**Keywords:** antiserum, tumor, antigens, hydatid cyst, passive immunization





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SYNTHESIS OF ECO-FRIENDLY SILVER NANO-PARTICLES FROM PENICILLIUM ACULEATUM AGAINST PROTOSCOLICES OF HYDATID CYSTS

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Hydatidosis as a helminth infection caused by the larval stage of the dog tapeworm Echinococcus granulosus is considered as a public health problem in different regions of the world. To date, scolicidal agents have been broadly applied for inactivation of the fertile cysts, which have several side effects on involved hosts. The objective of this study was to evaluate the scolicidal effects of synthesized silver nanoparticles (AgNPs) derived from the aqueous aerial extract of Penicillium aculeatum against protoscolices of hydatidosis in-vitro. Protoscolices were aseptically aspirated from sheep livers by having hydatid cysts. Various concentrations of green synthesis of Ag-NPs were used for 10-120 min, respectively. Viability of protoscolices was confirmed by 0.1% eosin staining. The findings showed that the Ag-NPs at all concentrations have strong scolicidal effects. The concentrations 0.1 and 0.15 mg/ml after 120 min of treatment showed 83% and 90% mortality rate, respectively.Current investigation indicated that applying biogenic Ag-NPs might be considered as a potential scolicidal agent in hydatidosis surgery, is less costly, safer, and requires non-toxic materials compared to existing chemical materials. However, further studies are required for the evaluation of efficacy of Ag-NPs in vivo.

Keywords: hydatid cyst, green silver nanoparticles, scolicidal, *Echinococcus granulosus* 

PREPARATION, PURIFICATION, CHARACTERI-ZATION AND ANTIPARASITIC ACTIVITY OF GOLD NANOPARTICLES: A BIOTECHNOLOGI-CAL APPROACH

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Intoduction & Objectives: Preparation of metallic nanoparticles (NPs) for pharmaceutical application by using biotechnological methods is an important area of research in nanotechnology. The present study proposed a green process for the extracellular production of gold NPs, which synthesized and stabilized using Penicillium aculatum isolated from soil. The synthesized gold NPs were characterized by using UV-Visible Spectroscopy, Fluorescence, SEM (Scanning Electron Microscopy), AFM (Atomic Force Microscopy), DLS (Dynamic Light Scattering), zeta potential, Polydispersity Index (PDI), Correlogram and Intensity fluctuations of NPs. The gold NPs were formed uniform with spherical shape and good monodispersity with the average diameter of 60nm.It was for the first time that scolicidal activity of biosynthesized nanogolds was assayed on hydatid cyst protoscolices of Echinococcus granulosus in vitro. It showed that biotechnologically prepared gold NPs may be considered as an effective scolicidal agent that would be applied for pharmaceutical application.

Keywords: gold nanoparticles, biosynthesis, antiparasitic activity, *Echinococcus granulosus*, scolicidal





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SEQUENCE ANALYSIS AND GENOTYPIC CHAR-ACTERIZATION OF ECHINOCOCCUS GRANU-LOSUS FROM HUMAN AND BOVINE ISOLATES BASED ON THE MITOCHONDRIAL CYTO-CHROME C OXIDASE 1 (COX1) GENE IN THE EAST AZERBAIJAN PROVINCE, NORTHWEST IRAN

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Hydatidosis is an emerging zoonotic parasitic disease caused by the larval stage of Echinococcus granulosus, which causes great health and economic losses. The aim of this study was to use the sequencing method to evaluate genotypes of E. granulosus isolated from humans and bovines using mitochondrial cytochrome c oxidase subunit 1 (cox1) gene. Overall, 26 hydatid cyst samples (10 human and 16 cattle isolates) were collected. DNA extraction was taken from the protoscolices of human and germinal layer of bovine samples. PCR was performed using the mitochondrial cytochrome c oxidase subunit 1(cox1) gene, and then it was sequenced. Sequences were analyzed for identification of their genotypes. All 16 bovine isolates were recognized as G1 genotype (sheep strain), and G1B subtype. Out of ten human host samples, seven isolates were G1B subtype, and three samples were identified as G3 genotype. The results of this study showed that G1, and especially G1B, are the predominant genotype and subtype in the East Azerbaijan province.

**Keywords:** *Echinococcus granulosus*, cytochrome c oxidase 1, human isolate, Iran

RAPID IDENTIFICATION OF ECHINOCOCCUS GRANULOSUS AND E. CANADENSIS USING HIGH RESOLUTION MELTING ANALYSIS BY FOCUSING ON SINGLE NUCLEOTIDE POLY-MORPHISM

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Echinococcus granulosus, causing hydatidosis in human and animals, is one of the most important zoonotic parasites worldwide. High resolution melting (HRM) analysis provides lowcost and sensitive scanning method for detection of variation in DNA sequencing. In this study, we have tested a HRM method for discriminating common genotypes of E. granulosus and E. canadensis. One hundred forty-one hydatid cysts were collected from slaughtered animals infected with hydatid cyst in different parts of Isfahan during 2013. After DNA extraction, the mitochondrial cytochrome c oxidase subunit 1 gene (cox1) was amplified using polymerase chain reaction (PCR) coupled with HRM curve. A result of the HRM analysis using partial sequences of cox1 gene showed that 93, 35, and 2 isolates were identified as G1, G3, and G6 genotypes, respectively. We found a single nucleotide polymorphism (SNP) in locus 9867. This position is a critical locus for differentiation between G6 and G7 genotypes. In phylogenic tree this isolate was located between the G6 and G7 genotypes, which suggests that this isolate may be the G6/G7genotype.As the G7 genotype has not been reported before in Iran, this intermediate genotype may suggest the existence of G7 genotype in this region. We believe that the HRM analysis that was developed in the present study provides powerful technique for molecular and epidemiological studies on E. granulosus in humans and animals.

Keywords: Echinococcus granulosus, cox1 gene, genotyping, HRM, Iran





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GENOTYPING OF ECHINOCOCCUS GRANU-LOSUS ISOLATES FROM SHEEP USING MITO-CHONDRIAL CYTOCHROME OXIDASE 1(COX1) GENE BY SEQUENCING METHOD IN TABRIZ

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Hydatidosis as one of the important zoonotic parasitic disease has posed many health and economic losses annually. So far, 10 genotypes (G1-G10) of Echinococcus granulosus species have been identified based on mitochondrial DNA analysis. This complex was divided to 4 species: Echinococcus granulosus sensu strict (G1-G3), E. equinus (G4), E. ortleppi (G5) and E. canadensis (G6-G10). Due to diversity and distribution of intermediate hosts in different parts of Iran and world, there are probably differences in parasite genotypes. Since the genotype study can be effective for providing vaccine to prevent infection transmission to intermediate hosts in geographic area, this study was conducted in order to Genotyping of Echinococcus granulosus isolates from sheep using mitochondrial cytochrome oxidase 1(cox1) gene by sequencing method in Tabriz. In this study, 19 sheep hydatid cyst samples were collected from the slaughterhouse of Tabriz. DNA was extracted from protoscolices of sheep samples. After PCR and electrophoresis, samples were sequenced and then were performed Genotypic and phylogeny analysis with the appropriate software. After genotyping, out of 19 sheep samples, one identified as G3 genotype (Buffalo strain), one as G1D and 17 samples as G1B subtype (sheep strain). The results of this study showed that the predominant genotype of Echinococcus granulosus is common sheep strain (G1 genotype) in East Azerbaijan province. Also G1B is dominant subtype in this region which indicates existence of dog-sheep circle.

Keywords: *Echinococcus granulosus*, sequencing, cytochrome c oxidase 1 (cox1), genotype, Tabriz

# A RETROSPECTIVE ANALYSIS OF HUMAN CYST-IC ECHINOCOCCOSIS IN ZANJAN AREA, NORTHWEST IRAN

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Hydatidosis is the most important zoonotic disease that causes significant economic losses and public health problems worldwide. In humans, it may range from asymptomatic infection to a severe disease and leads to death, and high treatment costs. This study was conducted to retrospectively evaluate 136 patients diagnosed with hydatid cyst disease at two university medical centers during 2007 and 2013 in Zanjan area, northwest Iran. We surveyed medical records of infected patients with hydatid cyst operated in two hospitals in Zanjan. Several parameters were studied including age, sex, place of residence, and the location of cysts. Of 136 cases, 54.4% were female and 45.6% were male with the mean age of 45 years (4–86). The most affected age group was 21-40 years old (36.02% of the cases). Cysts were localized in liver and lung in 64% and 23.5% of cases, respectively. Single organ involvement was seen in the majority of patients and 13 (9.5%) cases had multiple involvement. In 69.9% of cases there was only one cyst, 8.1% had two cysts, 17.6% with three cysts, and 4.4% had four cysts or more. The distribution of residence of patients showed that 33 (24.3%) of them were urban residents and 103 (75.7%) were rural. The results showed that the prevalence of hydatidosis is high in this city and further studies are required to determine the prevalence, economic impact and risk factors of the disease in the area.

Keywords: Zanjan, cystic echinococcosis, Iran





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# A RETROSPECTIVE ANALYSIS OF HUMAN HY-DATIDOSIS IN KHORASAN PROVINCE, A HYPER ENDEMIC AREA IN IRAN (MIDDLE EAST)

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Human cystic echinococcosis (CE), a zoonotic helminthic disease, is one of the most important health threatening in the world that imposes a large health and economic burden to governments. Like many countries in the words, human cases of hydatidosis are reported from Iran. The aim of this study was to analysis the medical records of operated patients as demographic data and laboratory findings. In a case series study, 473 medical records of operated hyadatidosis patients were analyzed during a 7 years period between 2001-2008 in Qaem hospital, Mashhad, Khorasan Razavi province, Iran. Data were analyzed using SPSS software and proper statistical methods. Out of 473 CE cases 255 (53.9%) and 218 (46.1%) were male and female respectively. Average age of patients was 36.95 and the majority of the patients were 20-29 years old. Eosinophilia rate in this study was 13.3% and just in monocytes and hematocrit variables statistically significant difference were found in different age groups. Diagnosis was done by serological and imaging techniques and using of imaging is more frequent than serological methods. Use of imaging techniques especially X ray, CT Scan and Sonography is more frequent than serological methods and MRI technique in brain and cord involved cases. Laboratory finding alone was not useful in diagnosis of CE and further surveys are needed to detect any probable correlation between hydatidosis and blood factors changes.

Keywords: cystic echinococcosis, CBC and WBC differential, blood factors, surgery, hydatidosis,

# VARIABILITY OF EMSB MICROSATELLITE MARKER AMONG FOUR TAENIDAE TAPE-WORMS ECHINOCOCCUS GRANULOSUS, TAE-NIA HYDATIGENA, TAENIA OVIS AND TAENIA MULTICEPS

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Larval stage (metacestode) of some taeniid tapeworms infects humans and ruminants as intermediate hosts and domestic and wild carnivores as the definitive hosts. Echinococcus granulosus, Taenia multiceps, Taenia ovis and Taenia hydatigena are among the most prevalent taeniid species of dogs in Iran. Epidemiological studies of canine echinococcosis are an essential element for implementation of hydatid control programs. The use of more sensitive tools such as microsatellites might provide more information about parasite DNA polymorphism; and is needed for transmission tracking studies. One of the suitable microsatellite markers that have been used so far is EmsB. The purpose of the present study was to determine the EmsB variability in four taeniid species infecting dogs in Iran. Twenty previously characterized isolates of each of the four Taeniidae tapeworms T. ovis, T. hydatigena, T. multiceps and E. granulosus were selected. PCR was set up with optimum conditions using EmsB marker with specific primers. All the PCR products were evaluated by 2% agarose gel and were visualized using ethidium bromide. We use 8% SDS PAGE for evaluating different patterns of PCR products in the tapeworms. SDS-PAGE results showed completely different banding patterns among the tapeworms. EmsB presented a specific pattern, with a notable difference among the isolates of Taenia species examined. Based on the results of the present study, different patterns of EmsB proved this microsatellite marker as a reliable tool for epidemiological studies on canine echinococcosis.

Keywords: EmsB, microsatellite, Taenia spp.





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TAENIIDAE INFECTION OF STRAY DOGS IN KERMAN, IRAN WITH SPECIAL REFERENCE TO SPECIFIC DETECTION OF ECHINOCOCCUS GRANULOSUS USING COPRO-PCR

#### <u>Seyedreza Mirbadie</u><sup>1</sup>, Mohammadali Mohammadi<sup>2</sup>, Saeedeh Shamsadini Lori<sup>3</sup>, Ebrahim Saedi Dezaki<sup>3</sup>, Hosain Kkamyabi<sup>3</sup>, Majid Fasihiharandi<sup>4</sup>

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Stray dogs play a key role in perpetuation of zoonotic parasitic infections. The significance and extent of Echinococcus infection of stray dogs in Kerman is not fully understood. The main objective of this study was to determine the prevalence of Taeniid parasites and specific detection of Echinococcus granulosus using copro-DNA PCR analysis in the stray dogs of Kerman city, southeast of Iran. Fecal samples from dogs were randomly collected within 24 h of defecation. All samples were transferred to the research lab, School of Medicine, Kerman University of Medical Sciences, Iran. The coprological examinations were conducted by both sugar flotation and formalin ether concentration methods. DNA extraction was performed using Qiagen DNA Stool Mini Kit according to the manufacturer's instructions. In microscopically positive samples, mitochondrial cytochrome c oxidase subunit 1 (cox1) specific primers were used to determine the Taeniid identity of the infection. In addition, another set of primers was used for the specific diagnosis of E. granulosus sensu lato. Totally, 307 fecal samples from stray dogs were examined for the presence of the parasites. Taeniid eggs were detected in 34 dogs (11.07%). All 34 taeniid positive specimens were PCR positive for cox1 (444 bp). Out of all taeniid positive specimens, 21 samples (6.84% of all dog specimens) were positive by E. granulosus specific primers. The findings of the present study revealed that canine echinococcosis is prevalent in the stray dogs in Kerman. The findings of the present study have important implications for hydatid control programs.

Keywords: Taenidae, fecal sample, copro-PCR, COX1

# COMPARATIVE IN VITRO EFFECT OF SILVER NITRATE AND HYPERTONIC SODIUM CHLO-RIDE ON PROTOSCOLICES OF HYDATID CYST IN A SHORT PERIOD, UP TO FIVE MINUTES

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Hydatidosis is one of the most important parasitic zoonoses. Surgery is the treatment of choice. Due to a risk of cyst rupture during surgery and the formation of secondary cysts, surgeons aspirate some of the cyst fluid before opening it and inject a protoscolicidal agent in to the cyst in order to prevent secondary cyst formation in the case of cyst rupture. However the application of these substances seems doubtful because the protoscolicidal effect of the substances and the necessary time for their effect are questionable and also most of these substances could create complications for the patients. Hence, we aimed to compare the protoscolicidal effects of silver nitrate 0.5% and hypertonic sodium chloride 20% in short periods up to 5 minutes. Cetrimide 0.5% with very high protoscolicidal effect as positive control and normal saline 0.9% as a negative control were also used in our tests. Materials and Methods: Sheep cysts of the liver and lung were collected from Shiraz slaughterhouse and were carried to the helmithology laboratory of Shiraz Medical School. A total of 3000-4000 protoscolices with a viability of over 90% were separately exposed to 1 mililiter of cetrimide 0.5%, hypertonic sodium chloride 20%, silver nitrate 0.5% and normal saline at different time periods of 1, 2 and 5 minutes. Afterwards, their viability was evaluated with eosin 0.1% and the obtained results were analysed using Anova and LSD methods. In vitro observations showed that the protoscolicidal effect of cetrimide 0.5% was 100% from the first minute and showed a significant difference (p<0.05), compared to other tested substances. At 2 minutes of exposure, the protoscolicidal effect of cetrimide 0.5% was also significant (p=0.003). At 5 minutes, there was no significant difference between cetrimide 0.5% and hypertonic sodium chloride 20% regarding their protoscolicidal effect (p=1), while at this exposure time, the difference between cetrimide 0.5% and silver nitrate 0.5%was significant regarding their protoscolicidal effect(p=0). The findings showed that hypertonic sodium chloride 20% at 5 minutes, had protoscolicidal effect; while silver nitrate 0.5% did not have a complete protoscolicidal effect up to 5 minutes.

**Keywords:** protoscolices, silver nitrate, hypertonic sodium chloride, protoscolicidal agents





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EPIDEMIOLOGICAL STUDY OF PATIENTS WITH HYDATID CYST IN EAST AZERBAIJAN PROV-INCE IN 2013-2014

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Hydatidosis caused by the larval stage of Echinococcus granulosus is one of the most important zoonotic parasitic diseases worldwide. Adult worms live in the small intestine of dogs and eggs excreted in dog feces. E. granulosus eggs ingested by intermediate hosts such as humans will cause hydatid cyst disease. Hydatidosis is prevalent in many areas of the world, and has special importance. The purpose of this study was to determine the epidemiology of hydatid cyst in the East Azerbaijan in 2013-2014. The profile of patients referred to health centers in East Azerbaijan province including sex, occupation, place of residence, history of contact with dogs, symptoms and treatment were studied and analyzed. From 19 patients, most cases were from Marand, 6 patients (31.57%) and Tabriz, 5 (26.31 %). Prevalence was similar in urban and rural areas. (63.16%) 12 cases were female and 7 cases (36.84%) were male. Infection of housewives (57.89 %) was higher than other occupational groups. 9 patients (47.36%) had a history of contact with dogs and 10 patients (52.64%) had not. The symptoms in 8 patients (42.10%) were abdominal pain (15.7%). In terms of treatment, the combination of surgery and drugswas the most common treatment and use of drugs and medication was the least.Since 63.16% of housewife patients were infected and had more contact with egg contaminated vegetables, it appears that contact with contaminated vegetawith should be considered as a major route of parasite transmission.

Keywords: hydatid cyst, epidemiology, East Azerbaijan

GENOTYPING OF HYDATID CYST ISOLATED FROM HUMAN IN KHORASAN PROVINCE

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Hydatidosis or cystic echinococcosis is one of the most important diseases in human and animals. Identification of strains is important for improvement of control and prevention of the disease. The aim of this study was to determine the strains isolated from human in Mashhad. 30 human hydatid cysts were collected from different hospitals of the province. Protoscolices were separated and their DNA genome was extracted by extraction kit. rDNA-ITS1 of each isolated samples was duplicated. PCR products were studied by electrophoresis and then were digested using Bsh1236I restriction enzymes. RFLP products were studied using electrophoresis on 3% agar gel. Result: Our findings confirmed that G1 is the dominant genotype of cystic echinococcosis in humans in eastern of Iran. This enzyme cut between CAnd G in 5'...C G^C G...3'. Totally 4 strains have been reported from Iran whereas in China, Kenya, Nepal and Argentina genotypes 1, 2, 3 and 6 have been reported, respectively. The results indicate the possiblity of transmission of the G1 genotypes of E. granulosusbetween livestock and humans in Khorasan province. Futher studies for detection of other strains in human is required.

Keywords: hydatid cyst, human, strain, Mashhad





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FERTILITY AND MORPHOLOGICAL CHARAC-TERISTICS OF HYDATID CYSTS IN CATTLE, SHEEP AND GOATS SLAUGHTERED IN SANAN-DAJ AND THEIR ROLE IN THE INFECTION OF THE FINAL HOST

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Hydatidosis is a zoonotic disease that causes decreased respiratory capacity, cough, edema, anemia and cachexia in the intermediate hosts (humans and herbivores) and finally leads to economic losses in the livestock industry. High levels of human infection is related to infected animals, therefore this study aimed to evaluate the fertility and morphological characteristics of hydatid cysts in cattle, sheep and goats slaughtered in Sanandaj and their role in the infection of the final host. Material & Method: In this study, 479 cattle, 1143 sheep and 329 goats were investigated and the number of cysts were counted separately in infected organs. Cyst fluid was drawn and measured and observed with light microscope for the presence or absence of protoscolex and the results were analyzed by statistical tests. From the total of 2051 investigated livestock, 51 cases (2.48%) were infected - 21 case were cattle (13 from lung, 8 from liver), 18 case were sheep (6 from lung, 12 from liver) and 11 case were goat (9 from lung, 2 from liver). The prevalence rate for cattle, sheep and goats were calculated 3.6%, 3.3% and 1.5%, respectively. In cattle, 0 cyst was fertile and 21 cyst were infertile, the mean volume was 19cc and the average number was 8. In sheep, 15 cysts were fertile and 3 cysts were infertile, the mean volume was 15cc and the average number was 6. In goat, 7 cyst were fertile and 4 cyst were infertile, the mean volume was 10cc and the average number was 6 observed in infected lung and liver.Due to the lack of fertile hydatid cysts in cattle, this animal is not likely to play a role in the cycle of the parasite and due to the fertility of cysts in sheep and goats these animals are probably responsible for the survival of parasites in the environment. Preventive measures should be implemented to control the disease, including treatment of guard dogs, eliminating the stray dogs and monitoring the slaughterhouses in term of health, sanitary measures, applying related standards and finally providing necessary public health training for breeders.

Keywords: hydatid cyst, *Echinococcus granulosus*, livestock, Sanandaj

# DETERMINATION OF EFFECTS OF FLUORIDE ON ECHINOCOCCUS GRANULOSUS PROTO-SCOLICES: AN IN VITRO STUDY

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The aim of this study was to develop a novel scolicidal agent adjunct to hydatid surgery. For this purpose, different molar ratios of CaF2 (5, 10 and 20%) were added to bioactive glass and their scolicidal activity was determined. The protoscolices of hydatid cysts were exposed to different masses (0.5, 1, 1.5 and 2 mg.ml-1) of bioactive glasses (with 5, 10 and 20% fluoride) for various time points (ranging from 1 min to 24 hrs) at 37 °C. The protoscolices were stained with 0.1% eosin and viewed under light microscope. The viability of protoscolices was determined by counting the living versus dead protoscolices. The scolicidal activity of the samples in all masses showed a significant increase in comparison with negative control after 5 min incubation (p < 0.05). Almost all the samples showed at least 50% scolicidal activity after 1 hr incubation time. According to our findings, 20%-FBG had around 100% scolicidal activity after 16 hrs in all tested masses. After 8 hrs incubation time in 2 mg.ml-1 weight mass, the BG samples with 20, 10, 5 and 0% fluoride showed 98±2, 93±5.8, 76.2±6 and 5.8±1.7% scolicidal activity, respectively.All the results obtained from this study strongly suggest that the scolicidal activity of the FBG depends on fluoride molar ratio, while this effect increased with the increase of the fluoride content in samples. It could be concluded that FBG has an effective protoscolicidal activity that makes it useful in prevention of secondary hydatidosis after hydatid surgery.

**Keywords:** *Echinococcus granulosus*, scolicidal, fluoridecontaining bioactive glasses, hydatidosis





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CHEMICAL COMPOSITION AND SCOLICIDAL ACTIVITY OF ZATARIA MULTIFLORA BOISS ES-SENTIAL OIL

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Zataria multiflora Boiss (Lamiaceae) commonly grows in Iran is a popular medicinal plant with various pharmacological activities mentioned in traditional Iranian medicine and modern phytotherapy. The present study was designed to evaluate the chemical composition and scolicidal effects of Z. multiflora essential oil on the protoscolices of hydatid cysts on an in vitro model. The components of the Z. multiflora essential oil were identified by gas chromatography/mass spectroscopy (GC/MS) analysis. To assess the scolicidal effects, protoscolices were aseptically aspirated from the naturally infected livers of sheep and goats. Various concentrations of essential oil were used for 5-30 minutes. Eosin exclusion test was used to determine the viability of protoscolices. The main components were thymol (41.81%), carvacrol (28.85%), and pcymene (8.36%). Findings showed that essential oil at the concentrations of 12.5 and 6.25µg/mL killed 100% protoscolices after 5 and 20 minutes of exposure, respectively. The results obtained in this investigation for the first time demonstrated that Z. multiflora essential oil might be a natural source for the production of new scolicidal agents for use against hydatid cyst surgery.

**Keywords:** cystic echinococcosis, prtoscolices, essential oil, thymol, carvacrol, GC/MS

# IMPROVED STRAIN IDENTIFICATION OF ECHINOCOCCUS SPP. USING PCR AND DNA SE-QUENCING OF COX1 AND ITS1 FRAGMENTS

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Human cystic echinococcosis, caused by Echinococcus spp., is one of the most important parasitic infections in Iran. To date, several genomic fragments have been used for identification and determination of haplotypes of these agents. However, it appears that a set of suitable genomic fragments could identify the relationship between phenotypic characteristics of the parasite with their genotypes. A total of 10 isolates of human, camel and sheep hydatid cysts collected from South Khorasan province were studied. Using PCR, mitochondrial nad1 and cox1, and ribosomal ITS1 fragments were amplified and then sequenced in both directions with the same PCR primers. After alignment, the sequences were compared with existing data in NCBI GenBank, and phylogenetic analysis was carried out. The sequence analysis of nad1 fragments indicated that the isolates belonging to G1and G6 genotypes. New primers to amplify Echinococcus cox1 region could sequenced 1200bp fragments, which in this study G6 samples most closely resembles to the isolates obtained in Mongolia (camel), Ethiopia (camel) and Russia (dogs and humans). Moreover, in the present study G6 isolates sequenced ITS1 fragments had a significant difference with the other E. canadensis (G7, G8 and G10) genotypes and could be differentiated between them.In the present study, the used primer sets showed a good sensitivity for amplifying cox1 and ITS1 fragments of Echinococcus. As regards by DNA sequencing, they can identify various strains of Echinococcus, their use is recommended in studies to determine the strain, haplotype and in phylogenetic analysis.

Keywords: genotype, cox1, nad1, ITS1, Echinococcus





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# PCR-RFLP PATTERN OF ANTIGEN B 2, 4 AMONG ECHINOCOCCUS GRANULOSUS ISOLATES IN TABRIZ, NORTH WEST OF IRAN

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Cystic hydatid disease (CHD) is an important parasitic zoonosis caused by infection with the larval stage of the tapeworm, Echinococcus granulosus. Despite the large number of studies, the parasitic antigens in hydatid cyst fluid (HCF) with immunodiagnostic value in detecting CHD are antigen 5 (Ag5) and antigen B (AgB). AgB is a suitable immunological marker for detection and progression of the disease. This study designed to find the polymorphism of B2, 4 subunite of antigen B among animal and human isolates. The result of this genetic variability is necessary and important for evaluation, application, and standardization of diagnostic tests using AgB purified from sheep hydatid cysts to detect specific antibody in human. 30 sheep hydatid cyst isolates were collected from domestic animals at slaughterhouses and 10 human isolates obtained from Imam Reza hospital. DNA was extracted from protoscolices of isolates and the gene loci related to Ag B2, 4 amplified with Eg Ag B2, 4 specific primer then the PCR products were digested with AluI restriction enzyme. All sheep isolates gave similar PCR-RFLP patterns after digestion with AluI (approximately 110 and 270 bp). The results of PCR-RFLP pattern indicate genetic similarity between human and sheep isolates highlighting the sheep strain is the most relevant strain related to humans infection. The comparative evaluation of the diagnostic performance of antigens showed that the antigen AgB had the best diagnostic performance. Therefore, genetic variability of AgB prepared from different E. granulosushosts in endemic regions must always be considered for evaluation of screening and diagnostic methods utilizing antigen B in order to detect specific antibodies.

Keywords: cystic hydatid disease (CHD), AgB, PCR-RFLP

# EPIDEMIOLOGICAL SURVEY ON PATIENT'S IN-FECTED WITH HYDATIDOSIS IN SHAHROOD DURING 2009- 2014

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Cystic hydatidosis/echinococcosis is an important zoonosis caused by the tapeworm Echinococcus granulosus. Severity of this disease depends on lifestyle, economic social and cultural conditions, water source, and hygiene practices. Cystic echinococcosis is considered endemic and is responsible for approximately 1% of admission to surgical wards in Iran. Hence, the present study aimed to have a glimpse at the present status of hydatidosis in Shahrood city during 2009-2014. Materials and Methods: In this retrospective cross sectional study, demographic data gathered from medical records during 5 years including sex, age, occupation, consumption of vegetables, numbers of cyst and infected organ. From 18 patients, 13 (72%) were male and 5(28%) female. Highest age of victim was 69 years and the least was 14 years. Prevalance in rural areas was significantly higher (5.9%) than the urban areas (1.2 %) (P < 0.001). Frequency of the disease among housewives (51.3%) was higher than other occupational groups. 61% of patients had two cysts and 22% three cysts. 88% performed simple washing to eat vegetables. Liver infection was seen in 60% of the patients with 1 case in lumbar spine. A definitive diagnosis was done with ultra-sound and CT scan. This result showed housekeepers as the most vulnerable group of hydatidosis. This might be due to more contact with raw vegetables. The rate of prevalence in this region was less than other cities of Iran like Mashhad or Esfahan.

Keywords: epidemiology, hydatid cyst, Shahrood, human





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Guilan, Iran; 20-22 May 2015Guilan, Iran; 20-22 May 2015COMPARING THE EFFECTIVENESS OF AL-<br/>BENDAZOLE AND SOLID LIPID NANOPARTI-<br/>CLES (SLNS) LOADED WITH ALBENDAZOLE ON<br/>DEVELOPMENT OF EXPERIMENTAL CYSTIC<br/>ECHINOCOCCOSISFERTILIT<br/>CYSTS I

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Human cystic echinococcosis (CE) is a zoonosis disease caused by the larval stage of Echinococcus (E.) remains a major medical problem in many parts of the developing world. Chemotherapy is the treatment of choice when surgery is not possible. The aim of this study was to determine the effects of free albendazole (ABZ) and ABZ loaded SLNs on in vivo development of CE and comparison of their effectiveness. ABZ-loaded SLNs and SLNs produced by high shear homogenization and microemulsion-emulsion congealing techniques. The nanoparticles were characterized in case of their mean size, morphology, drug loading capacity (DL%) and drug entrapment efficiency (EE %). Forty female Balb/c were randomly divided into four groups and infected by protoscolices. Control group: received only deionized water. SLNs group: received SLNs without drug. ABZ group and ABZ-loaded SLNs group received 50 mg/kg/day by gavage for three months. Two months after the last dose of the drugs, mice were sacrificed and their peritoneal cavity was opened for removal, counting, weighing and sizing of the cyst that had developed. ABZ chemoprophylaxis reduced the wet weight and size of developed cysts 56.3% and 57.7%, respectively. On the other hand, the corresponding results for the ABZ -loaded SLNs was 64.29% and 66%, respectively. Most of the cyst which developed in control and SLNs groups were hyaline (95.75% and 93.6%, respectively) which was higher than groups received free ABZ and SLNs loaded with ABZ (42.3% and 29.2%, respectively). Conclusions: We conclude that SLNs loaded with ABZ is more effective than ABZ alone in controlling the development of secondary hydatidosis in mice.

Keywords: albendazole, solid lipid nanoparticles, hydatidosis, *Echinococcus granulosus*, high pressure homogenization

# FERTILITY AND VIABILITY RATES OF HYDATID CYSTS IN SLAUGHTERED SHEEP IN KARAJ, IRAN

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Echinococcus granulosus is a small cyclophyllidean tapeworm (3-5 mm long) with an indirect life cycle. Wild or domestic canids are common definitive hosts and herbivorous or omnivorous mammals serve as intermediate hosts. Humans and livestock become infected through eating infective eggs excreted with dog feces. The aim of this study was to determine the fertility and viability of hydatid cyst in slaughtered sheeps. Two hundred hydatid cysts were collected from the liver and lungs of naturally infected sheep with E. grsnulosus when slaughtered in Municipal abattoir in Karaj, Iran. The collected cysts were analysed for rate of fertility and viability using parasitological methods. Out of the total of 200 cysts collected, (90%) were fertile and (10%) sterile. Fertility of the cyst was assessed by examining the cysts' fluid for the presence of protoscolices and the viability of the protoscolices was determined using staining with an aqueous solution of 0.1% Eosin. The rate of fertility in hepatic cysts and lungs cyst was 58% and 32%, respectively. The rate viability in the lung's cyst was 77.7% and the liver's cyst was 70%. It could be said that fertility rates of liver and lung hydatid cysts of sheep and viability of their protoscolices is considerable. This high rate of fertility and viability in sheep has an important role in completing the E. granulosus life cycle in this area. Therefore, safe disposal of infected carcass is necessary.

Keywords: hydatid cyst, fertility, viability, sheep





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# INVESTIGATION OF HUMAN CASES OF HYDA-TIDOSIS IN MAZANDARAN PROVINCE

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Hydatidosis, the disease caused by larval stage of Echinococus granulosus, can be regarded as one of oldest known parasitic diseases in the history of mankind. Echinococcus is also one of the smallest tapeworms of medical importance. The disease is prevalent in most of the temperate and tropical regions worldwide. According to the statistics, hydatid cyst accounts for 1% of all the surgeries in the hospitals of Iran. In this study, it was aimed to investigate the rate of hydatid cyst in the human population of Mazandaran during the past decade. Materials & Method: The research study was descriptive and retrospective and the data and information, which were available in the office of vice presidency for health, were utilized. The data were analyzed in terms of towns, site of the region, the risk factors and other epidemiological factors. The results of data analysis indicated that 69 individuals were infected with hydatid cyst - 2002 (7 persons), 2003 (3 persons), 2004 (2 persons), 2005 (7 persons), 2006 (6 persons), 2007 (7 persons), 2008 (3 persons), 2009 (2 persons), 2010 (12 persons), 2011(4 persons) , 2012 (4 persons), 2013 (8 persons) and 2014 (4 persons) . The highest rate of infection was found in Amol, 21(36.23%), and the lowest in Neka, Behshahr and Galugah which are located in the eastern part of the province. The source of infection was identified as contaminated raw vegetables in all the cases. The rate of infection was more among the women rather than men and abdominal pain was the symptom in most of the patients who referred to the health and clinical centers. Compared with other parts of the body, hydatid cyst was reported mostly in the liver, lungs and brain.Because of the temperate climate and abundance of stray dogs, as the parasite reservoir, in addition to their feeding on the viscera of animals resulting from unauthorized slaughtering, life cycle of parasites is completed and therefore there is high risk in the transmission of the disease. In the mountainous areas such as Amol the highest rate of infected cases was noticed. However, in the cities located in the plain areas (Neka, Behshahr and Galugah), the lowest positive cases were reported during the 13year period. Since, the disease can be classified as zoonosis, various departments including veterinary, environment and health and also other relevant organizations should be involved in control strategies.

Keywords: hydatidosis, mazandaran, human

SEROEPIDEMIOLOGICAL STUDY OF HUMAN HYDATIDOSIS BY ELISA METHOD USING RE-COMBINANT AGB IN DOROUD CITY, LO-REASTAN PROVINCE, WESTERN IRAN

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Hydatidosis is one of the most important and serious zoonotic disease throughout the world. In humans and animals it leads to health problems and the economical losses. According to the WHO classification, Iran is one of the endemic areas for the disease. The aim of this study was to conduct a sero-epidemiological survey in Doroud city, Lorestan Province, western Iran to detect the rate of hydatidosis in the city and nearby villages. 927 blood samples were collected using a cluster sampling procedure in 2013. Samples were analyzed using enzyme linked immunosorbent assay (ELISA) at medical faculty of Shahid Behshti University. 2 µg /ml antigens (recombinant antigen B), serum dilutions of 1:500 and anti human conjugate with 1:10000 dilutions were utilized to perform the test. Before sampling, a questionnaire was filled out for each case. Data were analyzed using Chi-square test. Out of 927 examined samples, 25 (2.6%) samples were infected with hydatidosis, which is equivalent to 260 person in 10000. The highest rate of infection was among 16-31 year old subjects (3.84%) and the lowest infection rate was seen in 31-45 year old individuals (1.14%). Surprisingly, there was no infection among ages over 60. According to statistics, there was no significant difference between the rate of infection and age groups. The rate of infection among males and females was 3.59% and 2.12%, respectively with no significant difference. The rate of infection in rural area (3.24%) was significantly higher than urban areas (1.2%). Among different occupation, farmers and ranchers showed the highest rate of infection (5.55%). The rate of infection with regards to academic level among different groups showed no significant difference. Infection in individuals that consumed unwashed vegetables (73.68%) was significantly more than those who consumed washed vegetables (1.21%). There are lots of contributing majors in the rate of infection to hydatidosis including: location, life style and hygienic principles.

Keywords: cystic echinococcosis, recombinant antigen, ELI-SA, Doroud





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COEXISTENCE OF ADULT WORMS, LARVAE AND EGGS OF CYSTOCAULUS OCREATUS WITH HYDATID CYSTS PROTOSCOLICES IN SHEEP LUNGS

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Cystocaulus ocreatus is a small lungworm that lives in the lung parenchyma and in subpleural nodules of small ruminants. The larval stage of Echinococcus granulosus or hydatid cyst affects ruminants's lungs, in addition to other tissues. In this study, coexistence of these two parasites in sheep is reported. In 2010, 90 hydatid cysts from sheep's lung were collected from a traditional slaughterhouse in Meshkinshahr, Ardabil province, Iran. During examination of the cysts, in two cases coexistance of a lung nematode with hydatid cyst protoscolices was found. Morphological and morphpmetric characterisations of the nematode were verified. Genomic DNA was extracted from larva and adult. Using PCR, ITS2-rDNA region were amplified; the PCR products were sequenced and the results compared with the sequences in GenBank. Both isolates were identified as C. ocreatus based on morphological features and the sequence of the nuclear ITS2 region. In one case, only alive C. ocreatus larvae and in the second case, alive egges, larvae and adult male and female were coexisted with protoscolices. Sequences of both isolate (KJ152179.1, KJ152178.1) were homologe, with 100% similarity with registered sequences of C. ocreatus in GenBank.Coexistence of alive eggs, larvae and adults of C. ocreatus with hydatid cyst protoscolices is a rare and interesting phenomenon. Apparently, chemical composition of hydatid cyst fluid is a suitable medium for development of C. ocreatus.

Keywords: Cystocaulus ocreatus, hydatid cyst, coexistence, ITS2

**COMPARISON BETWEEN THE EFFECTS OF AL-**BENDAZOLE AND MEBENDAZOLE ON THE **ENZYMATIC ACTIVITY OF EXCRETO-RY/SECRETORY PRODUCTS OF ECHINOCOC-**CUS GRANULOSUS PROTOSCOLICES IN VITRO

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Hydaticosis is caused by the larvae stage of Echinococcus granulosus. The cysts which are formed in human body can be treated clinically by surgery or chemotherapy using albendazole and mebendazole. Glutathione-S-Transferees (GST), same proteases and alkaline phosphatase (ALP) enzymes play important roles in detoxification, peptides proteolysis and protein synthesis of parasites respectively. The purpose of this study was to evaluate the comparison of the efficacy of albendazole and mebendazole on glutathiones-transferase, alkaline phosphatase and protease enzymes activities in protoscolices of hydatid cyst. Protoscolices were collected and cultured in RPMI1640 media at 37 °C, 5% CO2. The culture supernatants containing the parasite excretory / secretory (E/S) products were collected every 12 hours for 72 hours. The E/S products of treated samples with albendazole and mebendazole and the control one were collected and after centrifugation the protein concentrations were measured according to Bradford method. GST, ALP and protease activities of E/S products were assessed photometrically. In order to determine the statistically significant difference between E/S products of treated and control group, t-test was used. The mean of GST specific activity level in treated protoscolices with albendazole and mebendazole and in control group were obtained 69.44, 132.83, 225.47 U/mg/protein respectively. The mean ALP activity level in treated protoscolices with albendazole and mebendazole and in control group were 19.22, 22.27, 27.85 U/mg/protein respectively. The protease activity level in treated protoscolices with albendazole and mebendazole were not detected.While the mean of protease activity level in control group was 7.61/mg/proteins, statistical analysis showed significant difference between protein concentrations, the specific activities of GST, ALP and protease enzymes in treated protoscolices in comparison with control group (P<0.05). Also, the significant difference was seen in specific activities of GST and ALP enzymes in treated protoscolices with albendazole in comparison with treated group with mebendazole (P<0.05). The results show that both drugs have inhibitory effects on the activity of GST, ALP and protease enzymes. Albendazole is more effective on the enzymes activities (GST and ALP) as compared to mebendazole. This may by attributed to the different structures of the two drugs and might account for at least in part, difference in anti hydatid mechanism of these benzimidazole derivations.

Keywords: protoscolices, albendazole, mebandazole, protease, glutathione- s-transferase, alkaline phosphatase





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BACTERIAL DIVERSITY OF STERILE FLUID OF HEPATIC HYDATID CYST IN SINGLE-HUMPED CAMELS SLAUGHTERED IN INDUSTRIAL ABAT-TOIR OF QOM PROVINCE

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Hydatid cyst is the larval stage of the parasite Echinococcus granulosus in visceral tissues of its intermediate hosts. For unknown reasons, the penetration of bacteria into hydatid cyst fluid prevents the production of larvae or protoscolex of Echinococcus and cause sterility or non-sterile and fertile hydatid cyst. In this study, the bacterial diversity of sterile fluid of hepatic hydatid cyst in single-humped camels slaughtered in industrial abattoir of Qom province was examined. A total of 190 one humped camels were examined for the presence of hydatid cyst. 114 camels were infected. Cyst fluid of infected camels for bacterial diversity assessed and different bacteria identified using proper cultured media. The rate of hydatid cysts infection in one-humped camels slaughtered in industrial abattoir of Qom province was 60%. 95% of hydatid cysts, contaminated with bacteria and a variety of contaminants were including 22% of E. coli, 17% of Klebsiella, 15% of Proteus, 14% of Enterobacter, 11% of Staphylococcus aureus, 9% of non-hemolytic Staphylococcus, 8 percent of Pseudomonas and 4 percent Edward Silla.In this study, the prevalence and bacterial diversity of hydatid cyst in one-humped camels were assessed. Most of the bacteria cultured from fluid cysts were Staphylococcus aureus

Keywords: hydatid cysts, bacteria, single-humped camel, Qom

DESIGNING AND CONDUCTING IN-SILICO ANALYSIS FOR IDENTIFYING OF *ECHINOCOC-CUS* SPP. WITH DISCRIMINATION OF NOVEL HAPLOTYPES: AN APPROACH TO BETTER UN-DERSTANDING OF PARASITE TAXONOMIC STATUS

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The definitive identification of *Echinococcus* species is currently carrying out by sequencing and phylogenetic strategies. However, applying PCR-RFLP patterns are not broadly used as a result of heterogeneity traits of Echinococcus genome in different regions of the world. Therefore, designing and conducting a standardized pattern should indigenously be considered in under-studied areas. In this investigation, an in-silico mapping was designed for 8 Echinococcus spp. on the basis of conserved sequences of ribosomal DNA (rDNA) internal transcribed spacer 1 (ITS1) in Iran and the world. 60 Echinococcus isolates were collected from the liver and lungs of 15 human, 15 sheep, 15 cattle, and 15 camels' cases in Semnan province, central Iran. DNA samples were extracted and examined by polymerase chain reaction of ITS1-rDNA and PCR-RFLP via Rsa1 endonuclease enzyme. Moreover, 15 amplicons cytochrome oxidase 1 (Cox1) were directly sequenced in order to identify the strains/haplotypes. PCR-RFLP and molecular analyses revealed firmly presence of the G1 and G6 genotypes with heterogeneity of Cox1 gene although no other expected genotypes were found in the region. The finding shows that the identifying of novel haplotypes along with discrimination of Echinococcus spp. through regional patterns can illustrate the real taxonomic status of parasite in the central Iran.

Keywords: Echinococcus spp, in-silico, ITS1-rDNA, Cox1, G1, G6





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PREVALENCE, FERTILITY AND VITAL POWER OF HYDATID CYST IN SINGLE-HUMPED CAM-ELS SLAUGHTERED IN INDUSTRIAL ABATTOIR OF QOM PROVINCE

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Introduction and Objectives: Hydatidosis is a common disease of humans, animals, domestic and wild carnivores. In humans this disease cause varies injuries and sometimes leads to death. Hydatidosis in animals, especially livestock, cause great economic losses. Dogs, the reservoir hosts of the parasite, excreted eggs with the feces in the fields, contaminate water, plants and vegetables. Humans, who have history of close contact with dogs and consumption of contaminated water and vegetables and ruminants fed forage contaminated with parasite eggs, acquire hydatidosis. Hydatid cyst is a health and economic problem for countries where the disease is endemic. Hydatid cyst is an endemic disease in many parts of Asia, Europe, South America, Middle East, Australia and New Zealand. The disease has a worldwide distribution but the highest rate of infection is in the Mediterranean region, including Iran. Disease has been reported from all provinces of Iran. Among the intermediate hosts, sheep is the most suitable host and play a more important role in distribution of disease.Material and Methods: This review was done for a period of six months of 2013 (summer and autumn) in the slaughter health inspection at the industrial abattoir of Qom province. A total of 190 carcasses of one humped camels were examined. Sampling was performed from livers and the lungs of infected camels. In the laboratory, the cysts fluids were removed by the syringe and were collected in a glass, to determine fertile or sterile cysts by the macroscopic (sedimentation in the tubes) and microscopic (after centrifuge on coverslip) observations. In this study, the incidence of pulmonary and liver hydatid cysts were 60.5% and 46.3% respectively, and liver and pulmonary hydatid cyst fertility rates were 82.6% and 72.7%. Critical power of liver and lung cysts were determined 92.6% and 89.06% respectively. Discussions: The results obtained in this study seem to struggle with hydatidosis is essential and implementation of a program for controlling can have an important role in reducing infections. For example, the control of stray dogs prevented from releasing the thousands of eggs to the environment and with obliterate the infected offal in abattoirs, creating millions of adult worms in the small intestine of dogs is prohibited. After the implementation of a program to control of hydatidosis, what is important is the continuation of measures to safeguard the results obtained from implementing the program.

Keywords: hydatid cyst, camel, vital power, fertility, *Echinococcus granolosus*, Qom

PRELIMINALRY STUDY ON MOLECULAR CHARACTERIZATION OF ECHINOCOCCUS GRANULOSUS ISOLATES IN CHABAHAR, SISTAN AND BALUCHESTAN PROVINCE, IRAN BY COX1 GENE

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Echinococcus granulosus is the causal agent of cystic hydatid disease in man and animals. The aim of this study was to characterize the isolates of E. granulosus from sheep and cattel by sequence analysis of cytochrome C oxidase subunit I (COX1) mitochondrial partial gene. Martial & Methods: Two isolates of hydatid cysts, one from liver of sheep and other one from lung of cattel were collected from an abbatoir in Chabahar, Sistan and Baluchestan Province, Iran in 2014. The isolates were preserved in ethanol alchohol and transported to the Dept. of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences. DNA was extracted using a DNA kit from germinal layer and then the PCR amplification was performed for mitochondrial cox1 gene. The PCR products were sequenced after purification. The nucleotide sequences were edited by Chormas 2.4 software, and aligned by BLAST. Then, they were compared to those in the GenBank database. Result: Both E. granulosus isolates were determined as G1 strain. Their sequences had 99% homology with accession numbers KM 100573 and JQ250814 in GenBank.Similar to other parts of Iran, this preliminary study verifies the G1 genotype of E. granulosus as the probable dominant genotype in the study area.

Keywords: Echinococcus granulosus, COX1, G1 genotype, Iran





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IN VITRO EFFECT OF HYDROALCOHOLIC EX-TRACT OF MYRTUS COMMUNIS AGAINST PRO-TOSCOLICES OF HYDATID CYSTS

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Hydatidosis, a zoonotic disease caused by Echinococcus granulosus, is still endemic in many developing countries worldwide. Surgery is still one of the best choices for the treatment of the disease. The use of effective scolicidal agents during hydatid surgery is essential to prevent the secondary hydatidosis. Up to now no fully effective and safe agent has been identified for this purpose. It was shown that Myrtus communis has antibacterial, antioxidant and antiparasitic properties. In the present in vitro study, scolicidal effect of hydroalcoholic extract of Myrtus communis against protoscolices of E. granulosus has been investigated. Protoscolices of E. granulosus were aspirated aseptically from infected livers of sheep. Viability of protoscolices was evaluated by 0.1% eosin and the activity of flame cells. Hydroalcoholic extract of Myrtus communis in different concentrations (100, 50, 25, 12.5, 6.25, 3.12, 1.56 0.78 mg/ml) and at different exposure times (10, 20 and 30 minutes) was evaluated. Normal salin and silver nitrate were used as negative and positive controls, respectively. Concentration of Myrtus communis that kills 50% of protoscolices (LC50) was calculated by Probit analysis. The result of our study indicated that the highest scolicidal effect (100%) of Myrtus communis was obtained at concentratons 100 and 50 mg/ml. LC50 in 10, 20 and 30 minutes were 11.64 mg/ml, 7.62 mg/ml and 6.47 mg/ml, respectively. The results showed, Myrtus communis as a noble agent for inactivating protoscolices during hydatid cyst surgery. However, further studies are required for identification of the active ingredients in the plant extract.

Keywords: cystic echinococcosis, protoscolex, Myrtus communis, LC50 MORPHOMETRIC AND MOLECULAR CHARAC-TERIZATION OF ADULT ECHINOCOCCUS GRANULOSUS IN EAST AZERBAIJAN PROVINCE

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Hydatidosis, caused by metacestode of dog tapeworm Echinococcus granulosus, is considered to be a neglected cyclo-zoonotic disease in northwestern Iran. Although human hydatidosis is a high public health priority in the region, however there is lack of knowledge concerning the various features of adult E. granulosus in local stray dogs. The parasites were distinguished by morphometric diagnostic keys including small and large hook length, blade length, gravid proglottides and mitochondrial cytochrome oxidase 1 sequences. 16 (31.3%) out of 51 collected stray dogs were infected with E. granulosus. The measurement results of rostellar hook morphology and gravid proglottides unambiguously showed an intraspecies variation range among isolates (p < 0.05). Interestingly, the average ratios of blade length to total length in both large and small hooks were explicitly different compared with previous studies, which was conducted on metacestode scolices merely. The phylogenetic analysis of cox1 sequence revealed firmly the G1, G3, and G6 genotypes along with mixed infection. Based on analyzed sequences, 15 haplotypes were identified. This is the first simultaneous investigation, which is developed by employing both morphometric and molecular characterization on adult E. granulosus in Iranian stray dogs. It can reflect a better understanding of adult E. granulosus features, which should be considered precisely in taxonomy, biology, and monitoring of infected stray dogs in, northwestern Iran.

Keywords: Echinococcus granulosus, dogs, G1-G6 genotypes, morphometric, Iran





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IN VITRO EFFECTIVENESS OF MILTEFOSINE AGAINST PROTOSCOLICES OF ECHINOCOCCUS GRANULOSUS

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Cystic echinococcosis is one of the most common parasitic disease in human and animals which is caused by the larval stage of the Echinococcus granulosus. Surgical operation is the treatment of choice for hydatid cyst. During surgery many scolicidal agents, which have some complications, have been used for inactivation of the cyst contents, nevertheless some complications are caused by the scolicidal agents. Therefore, development of new agents with fewer side effects and more efficacies is necessary for effective treatment of the disease. Studies have shown cytotoxic effects of miltefosin on Leishmania Spp., Schistosoma mansoni, Trypanosoma cruzi and Entamoeba histolytica. The present study aimed to investigate the in vitro scolicidal effect of miltefosin. Protoscolices were obtained aseptically from the liver of slaughtered sheep at Kerman abattoir. Viability of protoscolices was evaluated by 0.1% eosin and the activity of flame cells. Protoscolices were exposed to different concentrations (1µM, 10µM, 100µM, 1mM and 10mM) of miltefosine at different times (10, 20 and 30 min). Normal salin and silver nitrate were used as negative and positive controls, respectively. Concentration of miltefosin that kills 50% of the total protoscolices (LC50) was calculated by Probit analysis. The result of our study indicated that miltefosine showed high scolicidal activity in vitro. The highest scolicidal effect (100%) of miltefosine was obtained at concentration 10 mM in 10, 20 and 30 exposure times. LC50 in 10, 20 and 30 minutes were 0.65 mM and 0.28 mM and 0.23 mM, respectively. The results showed, miltefosine is potentially a good candidate for disabling protoscolices during hydatid cyst surgery. However, additional studies are needed to identify the exact mechanism of miltefosine effect.

Keywords: miltefosine, protoscolex, hydatid cyst, LC50

# SEROEPIDEMIOLOGICAL STUDY OF HYDATID CYST USING AGB ELISA IN TORBAT-JAAM, KHORASAN RAZAVI PROVINCE, IRAN

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Hydatidosis is one of the most important zoonotic diseases and is found in all continents. Iran is one of the endemic areas and Khorasan Razavi is a hyperendmic region. The aim of this study was to determine the prevalence of human hydatid cyst by ELISA using AgB in Torbat Jam, Khorasan Rszavi province, where there is no information about human hydatid disease in this area, so far. A total of 1033 blood samples were collected randomly from Torbat Jam city and its 29 surrounding villages. Also Ag B was prepared from native hydatid cyst fluid of domestic sheep. Serum samples were tested by indirect ELISA while the suspected cases were re- tested. Mean+3SD optical density of negative samples was considered as cut off point. Result: From the total of 1033 sera collected for this study, 732 samples were female and 301 samples were male. The distribution of samples included, 668 rural and 365 urban samples. Fifty five (5.3%) samples including 43(5.9%) females and 12(4%) males were seropositive. In terms of living region, 40(6%) and 15(4.1%) of the samples were from rural and urban areas, respectively. Based on the source of drinking water, age group, literacy and job, the highest percentage of the cases were recorded among those who used the water fountain, their age range was 41 to 50 years, had a university education and were rancher.Result of this study showed a high sero-prevalence of hydatid cyst in this region. Elimination of stray dogs, treatment of domestic and sheepdogs, health education especially in the case of hydatid cyst should be done for prevention of the disease. Regarding Afghanistan as an endemic area of the disease, coterminous of the Torbat Jam, control of migration and migrants is essential for preventing.

Keywords: hydatidosis, ELISA, Torbat-Jaam





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# EVALUATION OF THE CONSTANT VIABILITY OF HYDATID CYST PROTOSCOLICES AT DIF-FERENT STORAGE CONDITION

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During the operation of hyadtid cyst, finding of the protoscolicidal materials with minimal side effects and more abilities is needed. To evaluate the effects of scolicidal agents, existence of the protoscolices with high viability and maintaining the stability of them is necessary in appropriate storage condition. The aim of this study was to examine the constant viability of protoscolices at different storage conditions. Protoscolices were gathered aseptically from slaughtered sheep livers and lungs in Qazvin abattoir. Protoscolices were divided in two segments, and one of them was kept in hydatid fluid and the other part was retained in normal saline after several times washing. Each one of the mentioned samples (rinsed and not rinsed), divided into two parts. Finally one part of them stored at the laboratory condition and the other one was kept at refrigerator temperature. The viability of liver and lung hydatid cyst protoscolices was assessed by 0.1% eosin. The highest viability rate of protoscolices and stability of them was related to the lung cysts in hydatid fluid and refrigerator temperature condition with 28.23 and 11.86 days, respectively. Also the lowest viability was about hepatic cyst protoscolices that were kept in normal saline and in laboratory temperature condition with 9.32 and 1.55 days, respectively. Differences of the viability rate of protoscolices and stability of them in lung and hepatic cysts stored in hydatid fluid and normal saline in refrigerator condition, was significant (p<0.001). The viability and stability of sheep's lung cyst protoscolices in cyst fluid and refrigerator temperature were better than other conditions. Therefore this method is suggested for researches on protoscolices.

# EVALUATION OF HYDATID CYST ANTIGENS IN DIAGNOSIS OF HYDATIDOSIS USING ELISA METHOD

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Hydatidosis is a chronic zoonotic parasitic disease with worldwide distribution and is considered as one of the most important parasitic infections. Different ways of diagnose the disease is progressing. The aim of this study was to evaluate recombinant antigens and hydatid cyst fluid antigens for diagnosis of human hydatidosis using ELISA method. In the present study the infected liver hydatid cysts were collected. Cyst fluid discharge and sediment were prepared for concentration. Then autoclaved liquid antigen, boiling liquid antigen and homogenized protosculex antigen were prepared. Also recombinant antigen was provided. Based on a statistical estimate the numbers of positive and negative serum samples of hydatid cysts were selected. Prepared antigens with the use of serums were evaluated using ELISA. The results showed that the sensitivity and specificity of the antigens were: autoclaved liquid antigen (69.4%, 34.31%), boiling liquid antigen (55.4%, 53.2%) and homogenized protosculex antigen (33.5%, 78.1%) and recombinant antigen (89%, 75.1%).In this study, recombinant and homogenized protoscolex antigens showed the greatest sensitivity and specificity, respectively. With further investigation using combination of two or more antigens we can develop diagnostic kits with higher sensitivity and specificity.

Keywords: recombinant antigen, ELISA, hydatidosis

Keywords: hydatid cyst, protoscolex, viability, temperature





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# IDENTIFICATION OF ECHINOCOCCUS SPECIES IN THE FECES OF DOMESTIC DOGS IN NORTH OF IRAN BASED ON MULTIPLEX PCR TECH-NIQUES

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Taeniidae is a large family of high medical and veterinary importance. Identification the eggs of this family in final hosts is not possible by morphological examination due to their closely similarity. Therefore, a multiplex polymerase chain reaction (PCR) was evaluated for the identification of morphologically indistinguishable eggs of the taeniid tapeworms after a sieving-flotation technique from domestic dogs using primers targeting mitochondrial genes. Fecal samples from 100 domestic dogs from north of Iran were submitted to flotation with zinc chloride for isolating parasite eggs using the flotation/sieving followed by multiplex PCR of mitochondrial genes. The mitochondrial multiplex reaction was designed to amplify a 395 bp fragment of NADH dehydrogenase subunit 1 (nad1) of Echinococcus multilocularis and 117 bp and 267 bp of a small subunit of ribosomal RNA (rrnS) of E. granulosus and other Taenia spp., respectively. Taeniid eggs were observed in 24% of examined dogs. With multiplex PCR method for detection of E. granulosus, Taenia spp. and mixed infection of E. granulosus, and Taenia spp. showed 12%, 10 % and 2%, respectively. Multiplex PCR revealed no E. multilocularis in examined samples. The prevalence of E. granulosus in domestic dogs as definitive hosts in north of Iran is high (14%), indicating the danger of acquiring this serious parasitic disease.

Keywords: *Echinococcus*, multiplex PCR, genotypes, domestic dog

DOMESTIC DOG AS A HUMAN HEALTH HAZARD IN NORTH OF IRAN

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Among the reservoir hosts of parasitic disease, dogs are the most important one. Several intestinal helminthic parasites such as Taenia spp., Dipylidium caninum, Toxocara canis, Hook worm, etc. can infect dogs. Therefore, an epidemiological study was conducted to determine the prevalence of helminthic parasites of domestic dogs using stool samples in north of Iran in 2013. Stool samples (n = 100) were collected from domestic dogs of different ages and genders. Different techniques including wet smear, formalin-ether sedimentation method and flotation technique in saturated zinc chloride solution were performed on collected stools to detect eggs and larval stage of helminthes parasites. The overall prevalence of helminthic parasites of examined stools of dogs was 57%. The examined domestic dogs harbored eggs of eight species of helminthes including: Taenia spp., Dipylidium caninum, Toxocara canis, ToxAscaris leonina, Capillaria aerophila, Mesostesphanus spp. and Trichuris volpis. T. canis and T. volpis were the most and least prevalent eggs, respectively. No significant statistical differences were observed between infection by helminthic parasites and both gender and age of the dogs (p>0.05).Considering high level of helminthic infection in the stool of examined domestic dogs, a continous educational program to increase awareness of dog owners and an effective anti-parasite control programm is highly recommended in order to reduce the danger of zoonotic diseases in north of Iran. This is the first report of C. aerophila and Mesostesphanus spp. from dogs in Iran.

Keywords: dog, zoonose, parasite, *C. aerophila*, *Mesostesphanus* spp, helminth, Iran





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*Guilan, Iran; 20-22 May 2015* HYDTID CYST DIAGNOSIS USING LAMINATED SERO-P

# LAYER ANTIGEN BY WESTERN BLOTTING

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Hydatid cyst is a zoonotic disease, which is common in most parts of the world and considered as a health problem in Iran. Differential diagnosis of this infection from other similar diseases is very important. Current laboratory methods for diagnosis of hydatid cyst are not sensitive or specific enough. Therefore, in this work diagnosis of hydatid cyst with different parasite antigens using western blotting technique has been investigated. Materials & Method: In this research, reaction of 15 sera from patient with hydatid cyst and 15 sera from normal people to hydatid cyst antigens has been investigated using western blotting. Different patients showed different reaction to laminated layer antigen of Hydatic cyst. However, it seems that the majority of patient reacted to one band.Considering this fact that laminated layer of hydatid cyst is in close contact to host tissue, more investigation on application of this antigen for diagnosis of this disease is recommended.

Keywords: hydatic cyst, diagnosis, western blotting

# SERO-PREVALENCE AND RISK FACTORS OF HUMAN HYDATIDOSIS IN CHAHARMAHAL VA BAKHTIYARI PROVINCE, SOUTH WEST OF IRAN USING ELISA METHOD, 2014-2015

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Hydatidosis caused by Echinococcus granulosus is an endemic zoonosis in most parts of Iran, including Chaharmahal va Bakhtiyari province. Local differences in prevalence and patterns of transmission of hydatidosis are affected by factors concerning the host, the strain of parasite, environment, and human behavior. This study was carried out to determine the prevalence of hydatid infection and associated factors in this region as one of the main poles of animal husbandry in the country. A total of 1280 out patients referred to different clinical laboratories of the province were included in this study. Serum samples collected from the subjects examined for specific anti-E. granulosus IgG antibodies by the Enzyme Linked Immunosorbent Assay(Elisa) kits purchased from Pishtaz teb company, Iran (sensitivity 91%, Specificity 96%). Demographic data were collected using questionnaire forms. The analysis of data was accomplished by the software SPSS ver.20 and Chi-square test and the logistic regression model. Out of the 1280 sera, anti-E. granulosus IgG antibodies were found in 26(2%) samples. Chi-square test showed a significant correlation between sero-prevalence of anti-E. granulosus IgG antibodies and style of living (tribal vs. rural and urban), location of residence (Lordegan county more than other counties), direct contact with dog, and household animal slaughtering (p0.05).Hydatidosis is endemic in this province and the main factors concerning the infection are style of living, residence, intimate contact with infected dogs, and household animal slaughtering. Therefore, it is recommended to improve the knowledge of population about risk factors and transmission route of the infection through health education programs.

Keywords: hydatidosis, *Echinococcus granulosus*, sero-prevalence, Iran





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# ANTIGEN AND ANTIBODY DETECTION IN URINE FOR DIAGNOSIS OF HUMAN CYSTIC ECHINOCOCCOSIS; A COMPARATIVE STUDY

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cystic echinococcosis (CE) is caused by the larval stage of cestode parasite Echinococcus granulosus with a worldwide distribution. The objective of this study was to compare the detection of hydatid cyst antigens and antibodies in urine of CE patients, using indirect ELISA, dot-ELISA and Sandwich ELISA. Urine samples were collected from 25 pathologically confirmed CE patients, 25 healthy controls and 32 non-CE patients. Using hydatid cyst fluid (HCF) of sheep origin as antigen, the samples were tested by dot-ELISA and indirect ELISA for detection of anti-hydatid cyst antibodies. In addition, cyst antigens were evaluated by sandwich ELISA. Cyst antigen was detected in 17 out of 25 (68%) of urine specimens using sandwich ELISA. Sensitivity of this test was calculated 68% and the specificity as 89.4%. Anti-hydatid cyst antibody was detected in 18 (72%) of urine samples with dot-ELISA techniques. Accordingly, the sensitivity and specificity of dot- ELISA method for detection of antibodies in urine samples were 72% and 86%, respectively. A sensitivity of 72% and specificity of 90.2% was calculated for indirect ELISA system for detection of antibody. The sandwich ELI-SA and dot - ELISA had reasonable performance in detection of hydatid cyst antigens and antibodies in CE patients and might be considered as a useful method in diagnosis of the CE patients. The results indicated that indirect ELISA was the most specific for diagnosis of hydatid cyst antibodies in urine.

Keywords: cystic echinococcosis, indirect ELISA, dot-ELISA, sandwich ELISA, urine

# IDENTIFICATION OF NOVEL POTENTIALLY MICROSATELLITE LOCI IN INTERNAL TRAN-SCRIBED SPACERS IN TAENIA SPP.: A COMPU-TATIONAL APPROACH

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In the field of molecular and epidemiological parasitology, characterization of fast evolving genetic markers appears as an important challenge to consider the diversity and genetic structure of parasites. Microsatellites are abundant unique tandem repeats of short (2-6 bp) DNA motifs in eukaryotic genomes. They are popular genetic markers and very useful for developing molecular and genetical studies. The tapeworm Genus Taenia comprises many different important species of tapeworm parasite infesting dogs, cats, humans, rodents and livestock animal species. Genes encoding ribosomal RNA and spacers occur in tandem repeats. Internal transcribed spacer (ITS) is widely used in taxonomy and molecular phylogeny because it is easy to amplify even from small quantities and has a high degree of variation even in closely related species. We tried to find potential loci of STR/SSR in ITS region via computational prediction. All the records of *Taenia* spices that have none-coding ITS complete sequence, downloaded from Nucleotide database from NCBI. After this, for prediction of potential microsatellites regions we used two softwares, GMATo v1.2 and PHOBOS v3.3.11 and all output results sorted and ranked. In an attempt to find prediction Microsatellites regions in Internal transcribed spacers of Taenia spp, we predicted 155 regions with a potential of microsatellite in ITS sequences.Some of these regions can be used as a prevision factor in intra assay or inter assay, for instance (GT)n, (GGTC)n and (TG)n, (CTGA)n respectively. For more validation of these regions, we recommend using the experimental methods.

**Keywords:** microsatellite, internal transcribed spacers, *Taenia* spp. computational prediction





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SEROLOGICAL RESPONSE OF IGG CLASS AND SUBCLASS ANTIBODY IN SERA OF MICE, SHEEP AND HUMAN AGAINST THE CATTLE CRUDE HYDATID CYST FLUID (HCF) ANTIGENS

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The existing diagnostic kit uses sheep cystic fluid antigen as an available antigen to evaluate the human IgG antibody. The current study was designed to evaluate the trace amounts of human, mice and sheep IgG antibody against the crude hydatid fluid antigens of cattle in order to evaluate different responses of these sera to cattle antigen, which is a potential antigen for screening the hydatidosis amongst these animals. 30 Balb/c mice were immunised with sheep hydatid cyst fluid antigens after using freunds adjuants. The required antigen were collected and prepared from naturally infected sheep and cattle slauthered from industrial slaughterhouse in Ilam, Iran. 30 positive samples sera from mice, sheep and human were used in ELISA as case and 30 healthy sera from each as the control. ELISA was employed to measure the IgG class and subclass responses. The statistic analysis tests were Anova and Post Hoc Analysis Model. The highest human IgG subclass response against cattle HCF antigens was IgG4 (0.82) while the lowest was IgG2 (0.26) as it was similarly against mice and sheep HCF antigens too. The best response was seen for mice IgG and IgG2b against cattle HCF. Anova analysis indicated a significant differences between IgG subclass responses to cattle HCF (P<0.001) in case group and for the human total IgG responses among case and control groups (P<0.01). The mean OD of mice IgG against cattle HCF antigen was 0.31 and the sensitivity and specificity of ELISA in case and control groups was 100% for both. The OD ratio was 3, 7.5 and 10 for sheep, human and mice IgG, respectively. Totally, there are some immune responses from the serum of each animal recognizing the crude hydatid cyst fluid antigen of the others, called crossreaction, by which we can specify the most appropriate antigens for diagnostic purposes. According to the results of the current study cattle HCF is appropriate candidate for such aims.

Keywords: hydatid cyst, crude antigen, IgG response, ELISA

# IN VITRO STUDY OF SCOLICIDAL EFFECTS OF PISTACIA ATLANTICA EXTRACTS ON PROTO-SCOLICES OF HYDATID CYSTS

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Use of effective scolicidal agents during surgery of cystic echinococcosis is essential to reduce the recurrence rate. The aim of the present study was to evaluate the in vitro scolicidal effects of hydroalcoholic extracts of Pistacia atlantica on hydatid cyst protoscolices. The hydroalcoholic extracts of Pistacia atlantica were used to investigate the scolicidal effects against hydatid cysts protoscolices. Echinococcus granulosus protoscolices collected from 50 infected livers of sheep with haydatid cyst. Various concentrations of plant extracts were used in different exposure times to viability assay of protoscolices. Among the extracts tested, fruit extract of Pistacia atlantica 0.1% (1.27  $\pm$  99.09) and leaf extract of Pistacia atlantica 0.1%  $(89.25\% \pm 18.42\%)$  have stronger protoscolices effect in 6 h, respectively. Mortality rate decreased with low concentration, and also increased with exposure times. The finding suggests that Pistacia atlantica might be used as a protoscolicidal agent in treatment of hydatid cyst.

Keywords: *Echinococcus granulosus*, *Pistacia atlantica*, protoscolicidal activity, hydatid cyst





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USAGE OF POLYETHYLENGLYCOL (4000) FOR ISOLATION AND PURIFICATION OF ECHINO-COCCUS GRANULOSUS ANTIGEN B (EGAGB) FROM HYDATID CYST FLUID

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Hydatid cyst, caused by the larval stage of the cestodes Echinococcus spp, is one of the zoonotic infections worldwide. Recently echinococcosis is classified under a group of neglected tropical diseases (NTDS's) by WHO. Echinococcus granulosus has a high prevalence in Iran due to the presence of various intermediate hosts in this country. National epidemiological studies show a rising trend of hydatidosis, so the diagnosis of this zoonotic parasitic disease is of great importance. Nowadays WHO has suggested using specific antigen specially antigen B (AgB) for serological diagnostics. AgB is a polymeric lipoprotein that disintegrates to 8.12, 16, 20.24 kDa subunits under reduction condition. In this article Polyethylenglycol (4000) for AgB isolation from Hydatid cyst fluid was used and evaluated in terms of its ability in AgB isolation. Finally, protein concentration of this antigen was measured by Bradford assay and confirmed by SDS-PAGE. The results showed the usage of the Polyethylenglycol (4000) as a thickener was so effective and led to more isolation of AgB, that hasn't been done so far. The results showed that concentration of AgB in hydatid cyst fluid was low and utilizing polyethylenglycol (4000) for concentrating HCF through decreasing volume of hydatid cyst fluid is recommended.

Keywords: antigen B, hydatid cyst, *Echinococcus granulosus*, isolation.

MOLECULAR AND MORPHOLOGICAL CHARAC-TERIZATIONS OF ECHINOCOCCUS GRANU-LOSUS FROM HUMAN AND ANIMAL ISOLATES IN KASHAN, CENTER OF IRAN

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Echinococcus granulosus is one of the most important zoonotic helminths worldwide. Different strains of the E. granulosus have been described based on morphological and molecular characterizations; however, there is scant information about phenotypes and genotype characteristics of E. granulosus in Iran. Hence, the present study was to evaluate the phenotypic and genotypic diversity of E. granulosusisolates collected from human clinical, sheep, goat and cattle based on morphometric characterization and using two mitochondrial genes, COX1 and NAD1, besides nuclear ITS gene in Kashan, center of Iran during 2012-2013. Morphometric characterizations were evaluated based on 19 standard morphometric parameters among 400 metacectode and analyzed by ANOVA and T-Test using SPSS software. To determine the fertility of protoscolices, the cyst fluids were subjected to morphological microscopic examinations. Protoscolices and endocysts were removed from each cyst and their total genomic, and fragments of cytochrome c oxidase subunit 1, NADH dehydrogenase subunit 1 mitochondrial genes (cox1 and nad1, respectively) and ITS rDNA were amplified by PCR and sequenced .Genotypic characterization was performed using PCR- RFLP amplifications of ITS rDNA, COX1 and NAD1 mtDNA and three different restriction enzymes HaeIII, Rsa1 and HpaII, respectively. Genotype diversity and sequence variation of the strains were studied by Bioinformatics software and in comparison with those mtDNA and rDNA sequences already deposited in GenBank. Finally, 27 samples from four hosts were sequenced for phylogenetic analysis and recorded in Genbank with accession numbers, ITS: KJ363920- KJ363928, ND1: KJ162552- KJ162560 and CO1: KJ162561- KJ162569. Biometric analysis of the nineteen characters showed that those 19 morphometric values of cattle isolates were significantly higher than human, sheep and goat isolates (P<0.05). Molecular analysis of CO1 and ND1 fragments of mtDNA as well as ITS fragment of rDNA obtained from G1 using PCR-RFLP technique with three restriction endonuclease enzymes was in agreement with the morphological findings. PCR-RFLP analysis using RsaI digestion demonstrated two bands of 230 and 320bp and using HpaII two bands of 110 and 304bp in sheep strain. Besides, PCR-RFLP HaeII demonstrated three bands of 70, 320 and 550bp in the same genotype. Genotyping and phylogenic analysis indicated that, all human, sheep, goats and cattle sequences located in one main branch on the phylogenetic tree, including E.granulosus. Compared with NCBI Genbank, all the DNA sequenced demonstrated 100% identity with E. granulosus and were identified as G1 (sheep strain) genotype. This study indicate that is only genotype G1 of E. granulosus circulates among human and the other intermediate hosts of this parasite in study area. Further studies with more sample size from different geographic areas of Iran are needed for E. granulosus mapping.

Keywords: *Echinococcus granulosus*, molecular phylogenetics, morphological characterization, genotype, PCR-RFLP, Iran.





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# SCOLICIDAL EFFECTS OF PISTACIA KHINJUK L. EXTRACT AGAINST HYDATID CYST PROTO-SCOLICES AND ITS ACUTE TOXICITY IN MICE MODEL

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This investigation aimed to evaluate the in vitro scolicidal effects of Pistacia khinjuk L. methanolic extract against protoscolices of hydatid cysts and also its acute toxicity in mice model. Protoscolices were aseptically aspirated from sheep livers harbouring hydatid cysts. Various concentrations of the essential oil (12.5-100 mg/mL) were used for 10 to 60 min. Viability of protoscolices was confirmed using eosin exclusion test (0.1% eosin staining). Twenty four male NMRI mice were used to assess the acute toxicity of P. khinjuk for 2 days. In addition, the preliminary phytochemical analysis of the P. khinjuk extract was carried out to determine the presence of tannins, saponins, alkaloids, terpenoids, phenols and glycosides in this plant. Obtained findings revealed that P. khinjuk extract at concentrations of 100 mg/mL after 10 min of exposure killed 100% protoscolices. Similarly, the mean of mortality rate of protoscolices after 20 min of exposure to concentration of 50 mg/mL was 100%. Furthermore, lower concentrations of P. khinjuk extract provoked a delayed protoscolicidal activity. The LD50 of the intraperitoneal injection of the P. khinjuk methanolic extract was 2.8 g/kg and the maximum non-fatal dose was 1.7 g/kg. The results of primary phytochemical screening demonstrated the presence of high amount of terpenoids, tannins, phenols, and lacking the alkaloids in this plant. The findings of present study demonstrated P. khinjuk extract potential as a natural source for producing new scolicidal agent to be used in hydatid cyst surgery, with no significant toxicity.

Keywords: hydatid surgery, scolicidal, protoscolices, toxicity

# GENOTYPING OF HUMAN AND ANIMAL LAR-VAL ISOLATES OF ECHINOCOCCUS GRANU-LOSUS USING MULTI LUCOUS SEQUENCE TYP-ING APPROACH IN KHUZESTAN AND TEHRAN PROVINCES

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Cystic echinococcosis is a zoonotic disease caused by larval stage of Echinococcus granulosus. E. granulosus contains several genotypes. These genotypes have high geographic diversity in Iran and worldwide. In the present study we aimed to characterize strain diversity and genotype variation of E. granulosus strains. Accordingly, multi lucous sequence typing (MLST) method, as one of the newest method for detection of similarities or differences among microbial pathogens was designed. Totally, 146 E. granulosus cysts from human and livestock were collected from Tehran and Khozestan provinces. Genotyping of these isolates was performed based on both cox1 and nad1 sequences. Gene diversity in some regions including nad1, cox1, atp6, act II and 12sr RNA, was determined by PCR sequencing method. Drawing of the phylogenetic tree and comparison of the results of genotyping and MLST was based on the trimmed concatenated sequences of the noted gene study bioinformatics softwares. Results of this study showed G1 genotype of the dominate genotype among both human (86.7%) and animal (78.6%) samples. Complete congruecny was seen for the identified genotypes based on nad1 and cox1 sequences. No significant association was observed between these genotypes and involved organs in studied samples (P>0.05). Investigation of the MLST results showed 72 STs among the studied isolates. The results of this study collectively showed high congruency between results of genotyping and phylogenetic analysis and proposed the newly developed MLST methods as powerful approach for disseminate of E. granulosus genotype and strains, which could be exploited as a tool for epidemiological studies.

Keywords: Echinococcus granulosus, cystic echinococcosis, genotyping, Khuzestan, Tehran





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## SCOLICIDAL EFFECTS OF ZIZIPHORA TENUIOR L. EXTRACT AT DIFFERENT CONCENTRA-TIONS AND EXPOSURE TIMES

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Hydatidosis is one of the most important zoonotic diseases and prevalent in many parts of the world. At present, surgery is the main treatment for hydatid disease. One of the most important risks of hydatid cyst surgery is the leakage of cyst contents and recurrence. Selection of the effective and safe scolicidal agents and injection of them into cysts can reduce the dissemination risk of viable protoscolices. Various scolicidal agents have been used for inactivation of the cyst content, but mostly are accompanied by adverse side effects. Currently the uses of medicinal plants as an alternative to synthetic materials, which are harmless, have been considered. The aim of this study was the evaluation of protoscolicidal effects of Ziziphora tenuior on the scolices of hydatid cysts. Hydatid cyst of the infected sheep livers were collected from the Qazvin abattoir and their protosclices were isolated under sterile conditions. Fertility and viability of protoscolices was confirmed prior to the experiments. The protoscolices were exposed with ethanolic extract of Z. tenuior, at different concentrations (3, 5, 10, 25, 50, and 100 mg/ml) and various exposure times (10, 20, 30, 40, 50 and 60 min). Sodium chloride 0.9% was regarded as negative control. Viability of protoscolices was evaluated by 0.1% eosin staining. Scolicidal activity of Z. tenuior extract on protoscolices at concentration of 3 mg/ml was 5.63% - 50.1% after 10 - 60 min. Also the efficacy of this medicinal plant at concentration of 5 mg/ml was 6.63% - 97.91% after mentioned exposure times. However, the extract at concentrations of 10 and 25 mg/ml killed 100% of protoscolices after 20 and 10 min, respectively. The results of present study showed that ethanolic extract of Z. tenuior has high scolicidal activity and could be used as an effective scolicidal agent.

Keywords: hydatidosis, Ziziphora tenuior, hydatid cyst, protoscolicidal, Iran EVALUATION OF THE PROTOSCOLICIDAL EF-FECTS OF ECHINACEA PURPUREA EXTRACT AT DIFFERENT CONCENTRATIONS AND EXPO-SURE TIMES

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Curently, surgery is the best method for the treatment of hydatid disease. Recurrence of the disease due to the dissemination of cyst fluid and protoscolices is the most important complications of surgery. Several scolicidal agents have been used for inactivation of the cyst contents, but most of them have serious side effects. Since Echinacea with a number of medicinal properties is one of the effective plants against a variety of infectious diseases, in this study the scolicidal effects of ethanolic extract of Echinacea purpurea was investigated at different concentrations and exposure times. Infected sheep livers with hydatid cyst were collected from Qazvin abattoir. The protosclices were isolated under sterile conditions and viability of them was examined and confirmed. Ethanolic extract of E. purpurea were exposed with protoscolices at 3, 5, 10, 25, 50, and 100 mg/ml concentrations and 10, 20, 30, 40, 50 and 60 min exposure times. Sodium chloride 0.9% was regarded as negative control. Viability of protoscolices was assessed by 0.1% eosin staining. The ethanolic effect of E. purpurea extract on protoscolices at concentration of 3 mg/ml was 13.33% - 39.20% after 10 - 60 min exposure times. Also the effectiveness of this medicinal plant at concentration of 5 mg/ml was 69.05, 75.88, 85.67 and 100% after 10, 20, 30 and 40 min respectively. Moreover, the ethanolic extract of E. purpurea at concentration of 10 mg/ml killed 100% of protoscolices after 10 min exposure time.Ethanolic extract of E. purpurea had a high scolicidal activity and might be used as an effective scolicidal agent. Further investigations for its possible side effects are suggested.

Keywords: hydatid cyst, hydatidosis, Echinacea purpurea, Iran





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## UNUSUAL STATUS OF DISSEMINATED HYDATI-DOSIS IN MAZANDARAN PROVINCE: A RARE CASE

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Human hydatid disease (HD) is a parasitic infection caused by the larvae of Echinococcus granulosus. Hydatidosis is still a major health problem in the infested areas of the world, mainly in sheep-raising areas. But owing to increased travel and tourism all over the world, it can be found anywhere, even in developed countries. It can involve any organ and its symptoms can be confused with almost any pathological condition. The liver is the most frequently involved organ (52%-77%) followed by the lungs (10%-40%) and Peritoneal (13%). Dissemination of HD may occur by leakage of cystic fluid due to rupture of the cysts into the peritoneal cavity. Peritoneal hydatid cysts are almost always secondary to hepatic involvement. The primary infection with E. granulosus is believed to occur during childhood. Thus, the clinical symptoms do not appear until several years after infestation. This study shows an unusual case of HD that last 48 years despite several medical interventions. HD is still public health concern in Mazandaran Province and should be considered. Case history: A 62-year-old female was admitted to the Razi hospital in Quaemshahr town with history of hydatid cyst in the liver, lung, spleen, brain, weight loss and decrease in hemoglobin and splenectomy. He was suffered from chronic pain in the waist and seizures. Part of the liver was totally removed. The hydatid sand was extracted from chest tube. The patient could not sit down and get up any more and she had spread pain in legs. Upon the increasing intensity of the pain over the 2.5 months, the patient was hospitalized again. As her condition, she died after 48 years suffering from HD.Hydatid disease of soft tissues is usually secondary to hepatic or lung involvement of the disease. The clinical symptoms are nonspecific and depend on the site of involvement, cyst size and the pressure caused by the enlarged cysts. Diagnosis of hydatid cyst cans be achieved by clinical findings, serological tests and imaging methods. Disseminated intra-abdominal hydatidosis is an infrequent condition that usually results from the rupture of a liver cyst, with subsequent seeding of protoscolices in the abdominal cavity. There is no standard treatment for this disorder; careful surgical removal of the cysts and prolonged medical treatment with albendazole with or without praziquatel are recommended. The determination of the definitive cause of death may depend on elucidating the histological features of non-apparent or equivocal macroscopic lesions. Sometimes Multi organ failures, Hepatic Encephalopathy and other defects can be the reason of the death. This clinical complication can hardly be diagnosed through routine autopsy procedures. In the present case, the patient died after 48 years suffering from HD despite good medical care. Because our country is an endemic area for hydatid disease, supplementary ancillary investigations in this field are needed.

Keywords: disseminated hydatidosis, *Echinococcus*, Mazandaran, albendazole

#### HIGH RESOLUTION MELTING ANALYSIS (HRM) FOR DIFFERENTIATION OF THREE MAJOR TAENIIDAE SPECIES IN DOGS, TAENIA OVIS, TAENIA HYDATIGENA AND TAENIA MULTI-CEPS

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Tapeworms of the genus Taenia include several species of considerable medical and veterinary significance. Accurate identification of these species in dogs is the prerequisite of any prevention and control program. Here, we have applied an efficient method for differentiating three major Taenniid species in dogs, T. multiceps, T. ovis and T. hydatigena. Highresolution melting (HRM) analysis is simpler, less expensive and faster technique than conventional DNA-based assays that enable us to detect PCR amplicons in a closed system. Set of 44 metacestode samples was used in this study. The species included 36 isolates of T. hydatigena, 4 T. multiceps and 4 T. ovis. All the isolates had already been identified by PCRsequencing and their sequence data were deposited in Gen-Bank. Real-time PCR coupled with HRM analysis targeting part of the mitochondrial cox1 gene was used to differentiate the Taenia species. Two distinct melting curves were obtained enabling accurate differentiation of T. multiceps and other two Taenia species. The HRM curves of Taenia multiceps and other two species were clearly separated at Tm of 79 to 80°C.In this work the efficiency of HRM analysis to differentiate three major Taenia species in dogs has been domonstrated using mitochondrial cox1 gene.

Keywords: Taenia, HRM analysis, cox1, melting curve





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## GENOTYPE DETERMINATION OF HYDATID CYSTS ISOLATED FROM ANIMAL AND HUMAN IN ILAM BY PCR-RFLP METHOD

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Introduction & Objective: Hydatidosis is one of the most important zoonoses caused by larval stage of Echinococcus granulosus, which is distributed worldwide. Determination of the parasite genotypes in relation to the host specificity and transmission routes is of great importance. So far, DNA based analysis of the isolates obtained from different host species has led to identification of 10 different genotypes (G1-G10) in the world endemic areas. This work was designed to identify the genotypes of the hydatid cysts isolated from domestic animals and people in Ilam. A total of 30 isolates, including 20 sheep isolates collected from industrial slaughterhouse, and 10 human isolates collected from hospitals were subjected to genotyping analysis using PCR-RFLP of the Mitoconderial DNA nad1 and cox1 region. A fragment of about 550 bp and 500 bp were amplified from all samples using nad1 and cox1 PCR. The endonuclease digestion of the PCR products resulted in the RFLP pattern that were the same for all isolates as the pattern expected for the sheep strain. The restriction patterns of all 3 enzymes used (AluI, HpaII and RsaI), consistently approved the same genotype. The PCR RFLP pattern obtained from our samples showed characteristics of Echinococcus granulosus sensu stricto, which is the same as what has been previously reported in Iran. It can be concluded that the hydatid cyst isolates in Ilam is basically similar to that of other endemic areas of Iran.

Keywords: hydatid cyst, genotype, Ilam, PCR-RFLP

## EVALUATION OF RECOMBINANT ANTIGEN AG EPC1 AND COPRO -AG FOR SERODIAGNOSIS OF CANINE ECHINOCOCCOSIS

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Diagnosis of Echinococcus granulosus in the definitive host, particularly in dog, is the significant complication in the endemic areas. Blood and fecal samples were collected from 3 treated puppies with 70000 protoscolices (>90% viability). In Addition, 16 blood and fecal samples were collected from dogs with natural infection and other carnivores intestinal worms and also 4 samples from naturally infected dogs just with E. granulosus. Sensitivity and specificity of dot -ELISA based on the recombinant Ag and copro- antigen for serodiagnosis of experimentally infected dogs was similar (100% and 91%, respectively). This result arises from the serum sampling which is collected from the dogs infected just with E. granulosus without any exposure to the other worms, whereas sensitivity and specificity of recombinant Ag for serodiagnosis of naturally infected dogs with E. granulosusand other carnivores intestinal worms was 100% and 75% also it was 100% and 50% for copro- antigen. Furthermore, sensitivity and specificity of recombinant Ag for serodiagnosis of naturally infected dogs just with E. granulosus was 100% and 100% also for copro- antigen was 100 and 50%, respectively. Moreover, efficacy of recombinant Ag was 87.5% and efficacy of coproantigen was 70.5%. As it was assumed, sensitivity and specificity of ELISA based on the recombinant Ag (for serodiagnosis of Echinococcosis in dogs) was higher than coproantigen. Copro- antigen detection revealed the presence of E. granulosus in the small intestine of hosts, while EpC1 showed positive result even in the dogs with previous infection. As previous studies have shown copro- antigen specificity was lower than the recombinant EpC1 antigen. As a matter of fact, if serological tests have specific antigen it can be more reliable. The ELISA test based on the Epc1 is less dangerous than copro- antigen production and it is possible to be provided as a rapid diagnosis kit. Despite all the advantages of recombinant antigens, copro- antigen efficiency is important for the detection of current infection of individual dogs with E. granulosus and for its control planning.

Keywords: E. granulosus, recombinant EpC1 antigen, coproantigen, sensitivity, specificity





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## THE EXTRACTION OF GARLIC DISTILLATE BY TWO METHODS OF CONSTANT AND MANTIS EXTRACTION AND ANALYSIS OF ITS EFFECTS ON HYDATID CYSTS PROTOSCOLICES

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Hydatidosis is one of the most dangerous contagious disease transmit from animals to humans. At the moment, the surgery and removal of hydatid cyst is one of the most decisive cures for humans, while the use of medicine is for subsidiary treatments. In compare with synthetic medicine, the discovery of herbal biological substances with antiparasitic effects snd without harmful side effects for humans is considered very important for controling and treatment of infectious diseases including hydatidosis. In this regard, Garlic is one of the herbs with medical use that contains antiparasitic effects. In this case, by taking in to account the pathophysiologic importance of hydatid cyst, the authors decided to study and analyse the influence of Garlic distillate on Hydatid cyst. Three Garlic distillate types of water distillate, alcoholic distillate, and water-alcoholic distillate have been made by two methods of using Soxhlet and Mantis method .Then by using High Performance Liquid Chromatography method, the amount of alicin in each sample was determined. The mortal effect of prepared Garlic distillates with high concentration and also concentration of 300 and 600 g/l on hydatid cysts protoscolices was investigated. The concentrated water distillate of Mantis resulted in killing 50% of protoscolices after one hour and 100% of them after three hours. The concentration of 300 and 600 g/l of the Garlic distillate killed all the protoscolices of hydatid cysts after 5 and 6 hours. The Soxhlet water distillate with high density could kill only 15% of protoscolices after 3 hours and after 9 hours kills the entire protoscolices of hydatid cysts. Densities of 300 and 600 g/l of the Soxhlet water distillate could not kill any of protoscolices after 7 hours.In garlic distillation process the use of solvent water Mantis method resulted in more alicin extraction in compare with Soxhlet method. The more amount of Alicin in the Garlic distillat, the higher the killing effect on the hydatid Cysts protoscolices.

Keywords: hydatid cyst, allicin, garlic extract, HPLC

## ASSESSMENT OF CROSS REACTIONS OF RE-COMBINANT EPC1 ANTIGEN FOR SERODIAG-NOSIS OF HYDATIDOSIS/ ECHINOCOCCOSIS

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Diagnosis of hydatidosis is mostly based on imaging techniques, while reliable tests with accepted sensitivity and specificity are still needed. Although recombinant antigens have been proposed in the literature, none has been systematically tested. In this study, the cross reaction of recombinant antigen EPC1 was evaluated. RNA was extracted and EPC1 gene was amplified and subcloned into the vector PET28a (+) and transformed into Escherichia coli BL21 (DE3) purified by Ni-NTA agarose Kit, and used to establish ELISA. Sera were collected from twenty human patients after surgical treatment, thirty sera from patients without surgery, and thirty sera from persons without any exposure to dogs. In addition, human sera from other parasitic infections, Toxocara sp., Strongyloides, Toxoplasma, Fasciola sp. were tested. Samples were collected from 3 experimental treated puppies, sixteen dogs with natural infection with E. granulosus and other carnivores intestinal worms (such as Toxocara sp., Mesocestoides, Dipylidium) and also 4 samples from naturally infected dogs just with E. granulosus. Sensitivity and specificity of recombinant Ag for patients after surgical treatment was 100% and 100%, for patients without surgery was 100% and 100%, and for individuals without any exposure to dogs 100% and 98% respectively. For other parasitic infections was 100% and 90%. For naturally infected dogs was 100% and 100%, and of naturally infected dogs with E. granulosus and other carnivores intestinal worms was 100% and 75%. Comparison of EPC1 with native and recombinant B-8 antigen showed not significant difference in the sensitivity and specificity. A B-8 antigen has higher specificity but has not been presumable for dog. EPC1 could be used for human and dogs with higher sensitivity and lower specificity. Native B antigen showed similar results but is not affordable. As in other studies, it should be used with a set of antigens.

**Keywords:** hydatidosis, echinococcosis, recombinant EPC1 antigen, recombinant B-8 antigen, native B antigen, serodiagnosis





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EPIDEMIOLOGICAL FEATURE OF ANIMAL HY-DATIDOSIS IN PAVEH, KERMANSHAH PROV-INCE

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Hydatid cyst is the larva of *Echinococcus granulosus* that is one of the intestinal parasites in dogs. Hidatidosis is the common parasitic disease in many countries in the world. Infection to hydatid cyst is one of the most important parasitic diseases in our country. This parasitic disease causes many economical and hygienic damages. The goal of this study was to investigate epidemiological condition of hydatidosis through determination of the inflection condition of slaughterhouses in Paveh city of Kermanshah province.In this study the infection conditions of the slaughtered animal in paveh city during 2011-2013 is described. Data were collected using a questionnaire and all of the data were analyzed using SPSS-21. According to collected data from 17190 sheeps and goats, 187 (1.08%) cases and from 2158 cattle, 42 (1.94%) cases were infected. The most cases of the hydatid cyst have been reported in liver organ in sheeps, goats and cattle. This study shows that cattle are more infected than sheep and goats with hydatid cyst. Liver is more infected than lungs. The average of infection has been reported in sheeps 10% and in cows 12% in Iran. This study calls for necessity of more attention to prevention and control of this zoonotic disease in animals in order to decrease economical damages and possibility of transmission of the disease from animal to humans.

Keywords: hydatid cyst, liver, lung, paveh

PREPARATION AND PURIFICATION OF ANTI-GENS OF HYDATID CYST

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Hydatidosis is a chronic zoonotic disease caused by the larval stage of *Echinococcus* worm with a worldwide distribution. In this study, crude and B antigens of hydatid cyst fluid were obtained to diagnose human hydatidosis using Counter Immunoelectrophoresis (CIEP), Enzyme Linked Immuno Sorbent Assay (ELISA), Dot-ELISA methods. Infected liver with hydatid cyst was collected from Tehran slaughterhouses to prepare cyst fluid in different stages. After extracting and purifying the cyst fluid, it was centrifuged using cold centrifuge then prepared to concentrate. The study also includes sera from hydatidosis (n=60), samples of worm parasites (n=55), fascioliasis (n=35) and toxocariasis (n=20) and negative control (n=35) were tested by CIEP, ELISA and Dot ELISA methods.Crude antigen of hydatid cyst indicated the specificity of 68.9% and sensitivity of 86.7% using CIEP method, and B antigen showed the specificity of 87.8% and sensitivity of 83.3% using the same method. Crude antigen of hydatid cyst showed the specificity 76.7%, sensitivity 93.3% using ELISA method, and B antigen showed the specificity 96.7% and sensitivity 88.3% using the same method. Crude antigen of hydatid cyst having serum dilution at 1:800 exhibited the specificity of 83.3% and sensitivity of 100% using Dot ELISA method and B antigen having serum dilution at 1:800 showed the specificity of 100% and sensitivity of 98.3% using the same method. The results of this finding showed that B antigen has the maximum specificity to diagnose hydatid test using Dot ELISA method.

Keywords: hydatidosis, antigen, ELISA, Dot ELISA, Serologic Diagnosis





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#### STUDY ON SARCOCYSTIS PARASITE IN SHEEP IN SARI BY METHOD OF J.P.DUBBEY

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Sarcocystosis is a protozoan and zoonotic disease. This parasite has a global outbreak and spreads by several species of the genus Sarcocystis. Although clinical signs are not observed in intermediate hosts, consumption of raw meat contaminated with these parasite cysts can cause human disease. This research was performed on sheep slaughtered In Sari. The purpose of this study was to evaluate the contamination of Mutton (flesh of mature sheep) exposed for sale by digestion method. For this purpose, a total of 100 sheep carcasses were sampled in Butchers in Sari. Samples were taken from the meat of the thighs and the diaphragm. The samples were analyzed by digestion method (J. P. Dubbey). Results obtained from this study indicated that 73% of the mutton, at least one of the two organs studied (Thigh - diaphragm) were infected with Sarcocystis. Research results indicate that high levels of mutton were contaminated with Sarcocystis. In addition, digestion method is reliable in comparison with the macroscopic method to check Sarcocystis in the carcass. Considering the high percentage of infection in mutton, care must be taken in cooking meat for deactivation of cysts.

Keywords: Sarcocystis, sheep, Sari, J.P.Dubbey method

## A SURVEY OF CRYPTOSPORIDIUM INFECTION IN SHEEP IN DIFFERENT REGIONS OF IRAN

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Cryptosporidium is an important zoonotic parasite in humans and animals worldwide. The main aim of this study was to investigate the prevalence of Cryptosporidium infection in sheep in different regions of Iran. Fecal samples (n=1,749) were collected randomly in asymptomatic sheep from different rural regions of Iran in 2011-2012. All samples were examined by using the cold modified Ziehl-Neelsen staining technique. Oocysts of Cryptosporidium were found in 11.3% (198/1749) of the samples. The infection rates in Hamadan, Esfahan, Yazd, Fars, Bushehr and Mazandaran provinces were 9.5%, 19%, 12.5%, 6.3%, 3.7% and 17.3% respectively. The rate of infection in animals under 1 year was 16.7% and the rate for male and female animals were 14.3% and 10.3% respectively. There was a significant statistical difference among Cryptosporidium infection, age groups (P<0.0001), and gender (P=0.02). This study is the first comprehensive report of Cryptosporidium infection in sheep in different regions of Iran. Therefore, further molecular studies are recommended in sheep to identify the source of contaminations (animals or humans) and designing control strategies.

Keywords: Cryptosporidium, Sheep, Iran





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## FIRST MOLECULAR IDENTIFICATION OF SAR-COCYSTIS OVICANIS (PROTOZOA, APICOM-PLEXA) IN THE BRAIN OF SHEEP IN IRAN

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The objective of the present study was to survey the presence of Sarcocystis in sheep's brain in North Khorasan province. In general, 80 samples of sheep's brain were collected from slaughtered sheep in slaughterhouses of North Khorasan. Tissue digestion method was used for observing bradyzoites in tissues. Histopathological processing tracing Sarcocystis and ensuing structural change in the brain tissue were conducted. PCR analysis was conducted on all the brain samples. Sequencing was done for one PCR product. Genotype was identified by Blast search and homology analysis. Sarcocystis spp. was found in one of the brain samples (1.25%) using tis sue digestion method. The presence of bradyzoite was also confirmed in the prepared histopathological sections. PCR analysis was positive in one of samples. Genotyping of one sample proved that Sarcocystis species was Sarcocystis ovicanis and the nucleotide sequence of this parasite was deposited in the GenBank database under accession number, KF489431. Sarcocystis ovicanis can involve brain tissue of sheep and consequently causes clinical symptoms.

Keywords: Sarcocystis ovicanis, brain tissue, sheep, Iran

## ISOLATION AND GENOTYPING OF ACAN-THAMOEBA STRAINS FROM WATER SOURCES IN BOJNORD CITY

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Acanthamoeba spp. are free-living amoebae commonly found in the environmental sources such as; soil, water and dust. This parasite is the causative agent of amoebic keratitis (AK). The objective of the present study was to investigate the presence of Acanthamoeba spp. in water sources in Bojnord City. In general, 50 samples of water were taken from different localities of Bojnord including agricultural canals, rivers, and swimming pools. Filtration and cultivation were carried out on non-nutrient agar medium (NNA). PCR analysis was conducted on positive samples. Sequencing was done for 10 PCR products. Genotypes were identified by Blast search and homology analysis. Data were analyzed by SPSS software. Acanthamoeba spp. was found in 34 (68%) of samples of water. Genotyping of 10 samples proved that Acanthamoeba belonged to T4 (100%) genotype. Acanthamoeba spp. are free living amoebae in our surrounding environment and we are exposed to them every day without being aware, however considering health principles is necessary for preventing the transmission of this parasite.

Keywords: Acanthamoeba, water, Bojnord





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EVALUATION OF CRYPTOSPORIDIUM INFEC-TION OF CHILDREN IN KOMIJAN CITY IN SPRING AND 2013

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Cryptosporidium spp. are intracellular protozoan parasite. Due to it's importance in inducing long term fatal diarrhea specially in immunocompromised or cancerous patients and also paying little attention to this parasite in most laboratories, this study were carried out. The purpose of this study was to evaluate Cryptosporidium infection of children in Komijan city. This study applied 150 stool samples from children under 8 years old brought to hospital of Komijan city. Samples maintained in potassium dichromate 2.5%, then transported to laboratory. After cleaning and concentration by sheather's method and preparing slides and staining by acid-fast staining, they were observed by ×100 objective. Resul:Of 150 samples, 4 (2.6%) were positive.Regarding the result of this study, contamination with Cryptosporidium can be an important cause of diarrhea in children under 8 years old, which necessitates more consideration in early diagnosis and control of Cryptosporidium infection.

Keywords: Cryptosporidium, potassium dichromate, acid-fast staining

PREVALENCE OF ANIMAL CRYPTOSPORIDIOSIS IN FARMS OF ILAM AND IDENTIFYING SOME ISOLATES BY PCR-RFLP

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Cryptosporidiosis is one of the most important parasitic infections in human and animals. This study was designed for survey on the prevalence of Cryptosporidium infection in farms of Ilam, west of Iran, using parasitology method and genotyping by Nested PCR-RFLP. Fecal samples of 217 cattle were collected fresh and directly from the rectum of cattle. All of the samples were examined by microscopic observation after staining with modified Ziehl-Neelsen (MZN). Genomic DNA extracted by using EURx DNA kit. A Nested PCR-RFLP protocol amplifying 825 bp fragment of 18s rRNA gene conducted to differentiate species and genotyping of the isolates using SspI and VspI as restriction enzymes. The prevalence of Cryptosporidium infection in cattle using both methods was 3.68%. Most of the positive cattle were calves under six months. Species diagnosis carried out by digesting the secondary PCR product with SspI that C. parvum generated 3 visible bands of 448, 247 and 106 bp and digested by VspI restriction enzyme generated 2 visible bands of 628 and 104bp. In this investigation, all of the positive samples were Cryptosporidium parvum. C. parvum (bovine genotype) detected in all positive cattle samples in Ilam, west of Iran. The results of the present study can help public health care systems for prevention and management of cryptosporidiosis in cattle and the assessment of cattle cryptosporidiosis as a reservoir for the human infection.

Keywords: Cryptosporidium, PCR-RFLP, prevalence, Ilam





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## EXPRESSION CRYPTOSPORIDIUM PARVUM GP40/15 GENE IN E. COLI

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Cryptosporidium - a widespread obligatory intracellular coccidian parasite- causes cryptosporidiosis in humans and animals and as an opportunistic parasite, can cause disability, severe illness and even death in individuals with impaired immune systems. There are no effective drugs to treat crytosporidiosis. A number of surface proteins of the parasite as target antigens in the immune response of the host is detected. The infection occurs by eating sporulated oocysts of different species of Cryptosporidium through oral - fecal rout. After being eaten, the sporozoites release from the oocysts and attack the microvilli of intestinal epithelial cells where starting the complicate life cycle of the parasite usually result in intestinal damages.In this process, sporozoite surface proteins play an important role. So the development of a vaccine against the disease is focused on the surface antigens. The antigen gp40/15 is regarded as an immunogen. In this study, the expression of coding gene for Cryptosporidium parvum proteins gp40/15 in E. coli bacteria was attempted to be used in future studies to investigate the immunogenicity in animals. In this study, Cryptosporidium parvum gp40/15 gene from a synthetic gene after optimization and verification transformed to E. coli BL21 cells and after the screening recipient cell from the recombinant plasmids, cell culture with IPTG induction and expression was evaluated by SDS-PAGE. The protein was confirmed by Western blot and purified by HPLC.Based on these results, it appears that the recombinant protein can be as a vaccine candidate for immunogenicity of animals and humans against Cryptosporidium parvum infection and it can be used to detect the gene.

**Keywords:** expression, gp40/15, *Cryptosporidium* parvum, *E.coli* 

## EVALUATION THE EFFECT OF PARASITIC IN-FECTIONS ON COGNITIVE FUNCTION IN VOL-UNTARY PERSONS IN SELECTED TEHRAN HOSPITALS

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Recent experimental evidences suggest that parasitic infections can invade stress system. Cognitive functions including sustained attention and short-term memory can be affected by stress and stress system. In the present research, cognitive functions of the protozoan infected people's attened at selected Tehran hospitals were evaluated by using PASAT software. 20 parasitic infected persons (27.17±2.72 years old) and 20 voluntary healthy persons (25.74±1.18 years old) were evaluated for their cognitive functions including sustained attention and processing speed using PASAT software. In addition, serum cortisol level was measured in 2 groups. Serum cortisol concentration was significantly higher in infected persons. Sustain attention in infected group was significantly lower compared with control group. Correct answers to questions mean time was significantly lower in comparison with the control group but incorrect answers mean time significantly was higher than control group. It seems that parasitic infections can evade stress system and it can lead to reduced brain cognitive functions including sustained attention as well as processing speed.

Keywords: parasitic infections, serum cortisol, cognitive function





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CRYPTOSPORIDIOSIS: PREVALENCE, RISK FAC-TORS, AND SYMPTOMS ASSOCIATED WITH GAS-TROINTESTINAL DISORDERS IN PATIENT IN NAHAVAND COUNTY, WEST OF IRAN 2014

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Cryptosporidium is small microscopic parasite classified as an emerging pathogen. It is distributed worldwide, associated with enterocolitis, and has emerged as an important origin of diarrhea, mainly in children and in immunocompromised individuals. The aim of this study was obtaining the frequency of Cryptosporidium in patient with gastrointestinal disorders in Nahavand city of Hamadan province. A total of 1301 stool specimens during spring and summer, 2014 were collected from patient with gastrointestinal disorders suffering from abdominal pain, crumping, bloating, vomiting, nausea, diarrhea, dysentery and constipation. Information and symptoms of each patient was recorded in a questionnaire. The stool samples were stained with Zeil-Neelsen method after formaldehyde - diethyl ether concentration. Mucoidal stools were stained directly. The results were analyzed using SPSS version 16. Cryptosporidium oocysts were found in 17 (%1.3) out of 1301 stool specimen of the patients- from 683 (52.5%) male and 618 (47.5%) female patients. Of these 13 (2.62%) of children  $\leq$  12 years old age were infected while only 4 (0.49%) of the ages more than 12 years were infected. The prevalence of Cryptosporidium in summer (1.83%) in comparison to the spring (0.55%) was higher. Cryptosporidium is a common, zoonotic parasite that is highly prevalent in developing countries and leading cause of persistent diarrhea, vomiting and nausea signs especially in children. This coccidian was more common in children  $\leq 12$  years old ages. Significant association was observed between cryptosporidiosis and clinical signs such as diarrhea, and also significant relation was observed between the prevalence of cryptosporidiosis and season.

Keywords: Cryptosporidium, risk factors, gastrointestinal disorders, Nahavand, Iran.

## PRENVALENCE OF SARCOCYSTIS IN SLAUGH-TERED SHEEPS IN KHORRAMABAD BY MACRO-SCOPIC OBSERVATION AND PCR METHOD

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Genus of Sarcocystis comprises of protozoan parasites belongs to phylum Ampicomplexa that have reported from all around the world. This parasite has obligatory dixenous life cycle. Herbivoures (as intermediated hosts) will infect via ingesting contaminated water and food with sporocysts shed from carnivoures (as definitive host) and tissue cysts will be formed subsequently. Observation was performed from 500 slaughtered sheep. Fifty grams from esophagus, diaphragm, intra-costal, abdomen and biceps muscles were sampled. Extraction of DNA was performed by traditional kit and to replicate the 18s rRNA PCR was prepared. In addition, DNA from Neospora caninum and Toxoplasma gondii were considered for determining the specificity of primers. In direct observation 92 cases (18.4%) were macroscopic cysts. And from 100 randomly taken samples which were apperantly negative, PCR revealed 100% microscopic cysts.Due to the high prevalence of this parasite in the study area, survey on routes, control and treatment of infection of Sarcocystis seems to be necessary.

Keywords: Sarcocystis, PCR, sheep, Khorramabd





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#### MOLECULAR IDENTIFICATION OF SARCOCYST-IS SPECIES IN SLAUGHTERED SHEEPS IN BA-BOL BY PCR-RFLP

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Genus of Sarcocystis is a protozoan parasite belongs to Phylum apicomplexa, which has reported from all around the world. This parasite has obligatory dixenous life cycle. Herbivoures (as intermediate hosts) will be infect via ingesting contaminated water and food with sporocysts shed from carnivoures (as definitive host) and tissue cysts will be formed subsequently. The aim of the presented study was to determine the macroscopic Sarcosystis species in slaughthered sheep in Babol by PCR-RFLP. In the present study, totally 30 tissue samples from diaphragm, intercostal sternum, tongue and striated muscles of slaghthered sheep were collected. Extraction of DNA was performed by traditional kit and to replicate the 18s rRNA, PCR was prepared. Also, DNA from Neospora caninum and Toxoplasma gondii were considered for determining the specificity of primers. PCR-RFLP revealed that detected macroscopic Sarcocysts belonged to S. gigantea. This study showed that PCR-RFLP can distinguish sheep Sarcocysts and TaqI and HincII enzymes and is more effective for discriminating the macroscopic cysts.

Keywords: Sarcocystis, sheep, PCR-RFLP, Babol

SEROPREVALENCE OF NEOSPORA CANINUM IN NATIVE CATTLE IN KURDISTAN PROVINCE, WEST OF IRAN

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Neospora caninum is a worldwide-distributed pathogen which causes abortion in cattle leading to economic loss in the cattle industry. The aim of this study was to determine the seroprevalence of N. caninum antibodies in the native cattle slaughtered in various areas of Kurdistan province, from September 2010 to September 2011. Serum samples from 368 cattle slaughtered in seven slaughterhouses in this region were taken to detect anti-N. caninum antibodies using commercial N. caninum ELISA kit. Antibodies to N. caninum were found in 29 samples (7.80%). Regarding to different age groups, the prevalence of anti-N. caninum antibodies was reported 9.8% in 8 yr-old (P=0.895). There was no significant statistical difference in the prevalence of abortion between seropositive and non-seropositive cattle (P=0.588). The present study was the first report of Neospora infection in this region and indicated that native cattle of Kurdistan province were exposed to this parasite.

Keywords: seroprevalence, ELISA, Neospora caninum, cattle, Kurdistan, Iran





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## MOLECULAR IDENTIFICATION AND CHARAC-TERIZATION OF CRYPTOSPORIDIUM SPECIES FROM DAIRY CALVES BY NESTED PCR-RFLP IN ISFAHAN PROVINCE, IRAN

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Cryptosporidium spp. are common intestinal pathogens with wide distribution in young animals and humans. Various species of Cryptosporidium were detected in both hosts. The aim of this study was to determine the prevalence of various genotypes of Cryptosporidium spp. in calves' fecal samples in different husbandry in Isfahan province using nested PCR-RFLP method. One hundred fecal samples were collected from calves less than 8 weeks old in Isfahan husbandries. Fecal samples were investigated via Sheather's flotation and modified Ziehl-Neelsen staining methods. DNA was extracted from positive samples and Nested PCR followed by restriction fragment length polymorphism (RFLP) by VspI and SspI endonuclease enzymes. Result: Overall 33 (33%) of studied calves were microscopically infected with Cryptosporidium spp. which were all confirmed by nested PCR too. Rate of Cryptosporidium infection was more in cattle less than four weeks old than those between four to eight weeks old (48.8% vs 21.1%, P= 0.005). Also infection was more seen in cattle with diarrhea than those without symptoms (43.6% vs 20.0%, P= 0.018). All isolates except two (Cryptosporidium hominis) were identified as Cryptosporidium parvum. This study confirmed the relevance of C. parvum as a major etiology of cryptosporidiosis in dairy calves and showed its broad distribution in the area. Given that C. parvum is a zoonotic species, calf managers should be considered as those who are broadly exposed to the infection.

Keywords: Cryptosporidium spp., calves, Nested PCR-RFLP, Isfahan

## PREVALENCE OF CRYPTOSPORIDIUM SPP. IN DAIRY CATTLE IN MASHHAD, IRAN

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Cryptosporidiosis is a zoonotic disease caused by the Apicomplexan intracellular, extracytoplasmic coccidian parasite of the Genus Cryptosporidium which can infect a wide range of animals, including man. C. parvum also causes disease in humans and is, therefore, a zoonosis transmitted from cattle to humans but in immunocompromised individuals such as children and patients with Acquired Immunodeficiency Syndrome (AIDS), the infection fulminates and might be life-threatening. The study populations comprised 200 calves aged ≤6 months, randomly selected from 25 smallholders dairy and traditional managed herds, respectively. Presence of *Cryptosporidium* spp. oocysts in feces samples was detected using the modified Ziehl-Neelsen staining technique.Among the 200 stool samples of children examined, 6% showed oocysts of Cryptosporidium spp.Since, the prevalence of cryptosporidiosis in animals and herds was high the disease should be considered endemic and locally widespread. Thus, health education, avoiding of drinking contaminated water and hand hygiene following contact with domestic animals can protect the children of being infected with Cryptosporidium.

Keywords: Cryptosporidium, cattle, Mashhad





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STUDY OF THE PREVALENCE OF INTESTINAL PARASITES (PARTICULARLY CRYPTOSPORIDI-UM) AMONG DIARRHEAL CHILDREN ADMIT-TED TO DEPARTMENT OF PEDIATRICS OF HEFDAH-SHAHRIVAR HOSPITAL IN GUILAN

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Cryptosporidium is recognized as an important widespread cause of severe and prolonged diarrhea mainly in immunocompromised or malnourished children, but there is no attention to identifying the parasite. For first time in Rasht, the present study was designed and undertaken to assess the prevalence rate of parasites agents causing diarrhea particularly Cryptosporidium in patient hospitalized in children ward of Hefdah-Shahrivar hospital. Material & Methodes: In this cross-sectional study, from 2013 to 2014, stool samples collected from 42 children with diarrhea admitted to children ward of Hefdah-Shahrivar hospital. To identify parasites, direct smear, formalin-ether and modified acid fast staining techniques were applied. All samples were negative for Cryptosporidium and other parasite cysts or Ova. This study showed that non-parasitic agents were important causes of diarrhea. The reason of apparently negative results for Cryptosporidium was due to low prevalence of these parasites. As there is no accurate information about this parasite in Guilan, to determine the role of parasites especially Cryptosporidium as a cause of diarrhea, it is necessary to design another study using higher number of samples particularly children and immunodeficient subjects in Guilan.

Keywords: diarrhea, children, parasite, prevalence, Cryptosporidium STUDY OF INTESTINAL MICROSPORIDIOSIS AMONG KIDNEY TRANSPLANT PATIENTS IN HAMADAN, WEST OF IRAN

## Zahra Gharibi<sup>1</sup>, Mohammad Fallah<sup>2</sup>, Amir Hossein Maghsood<sup>3</sup>, Masoud Saeedi Jam<sup>3</sup>, Farahnaz Dadras<sup>4</sup>, Gholam Reza Hatam<sup>5</sup>

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Organ transplantion and using immunosuppressive drugs continuously are major risk factors for opportunistic infections that sometimes result in severe conditions that constantly must be monitored. Microsporidia is an obligate intracellular parasite and one of the important agents causing chronic diarrhea in immunocompromised patients. The laboratory diagnosis of organism is relatively difficult and chromotrope staining is a standard diagnostic method for detection of microsporidia. The aim of the present study was to determine the frequency of intestinal Microsporidia infection among kidney transplant patients. Matrials & Methods: A total of 180 stool specimens were taken from kidney transplant patients admitted to Beheshti educational hospital of Hamadan. The patients were using the immunosuppressive drugs continuously. The patients selected with or without gastrointestinal symptoms. The modified trichrome staining and calcofluor white was used to identify Microsporidia in stool samples. Air dried, methanol fixed thin stool smears prepared and stained by modified trichrome staining and calcofluor white, then slides examined using light microscopy. Also stool samples were tested by PCR. Totally 86 patients (47%) were female and others were male. The mean duration of kidney transplants and immunosuppersive treatment was 5.5 years. Only one (0.55%) of 180 patients was positive for Microsporidia and she was a female patient. This 45-year-old patient live in Hamadan, kidney transplant surgery was done about 10 years ago, and her main gastrointestinal symptom was diarrhea and recieved cyclosporine and prednisolone. Conclusions: Based on the frequency of this opportunistic infection, fortunately, this parasite is not a common infection in these immunedeficient patients despite using long time immunosuppressive drug.

**Keywords:** Microsporidia, modified trichrome staining, calcofluor white, PCR, kidney transplantation





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## MOLECULAR CHARACTERIZATION OF MACRO-SCOPIC SARCOCYSTIS SPP ISOLATED FROM SLAUGHTERED SHEEPS IN DORUD COUNTY

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Sarcocystiosis caused by different *Sarcocystis* species, is a zoonotic parasitic infection that infects human and a wide range of animals. In this study, investigation of *Sarcocystis* infection was performed in slaughtered sheep in Dorud city using 18S rRNA gene and restriction enzymes. For this purpose, macroscopic cysts were collected from esophagus, diaphragm, intra- costal muscles, then DNA was extracted by Commercial Cinagene Kit and the gene was replicated and finally by Eco RI, Taq I, Ava I, Hind II enzymes, the replicated gene was restriced. All of the specimens was 100% *Sarcocystis gigantea*.According to high prevalence of this parasite in the study area, survey on prevention routes, control and treatment of the infection seems to be necessary and also for differentiation of macroscopic species, Taq I, HindII were more useful than others.

Keywords: Sarcocystis gigantea, sheeps, Dorud

MOLECULAR IDENTIFICATION AND RISK FAC-TORS OF CRYPTOSPORIDIUM INFECTION IN CHILDREN UNDER 10 YEARS, REFERRED TO THE HEALTH CARE CENTERS OF HAMADAN DISTRICT, 2013

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Cryptosporidium, one of the most important zoonotic intestinal protozoa, can cause gastroenteritis in immunocompromised patients and children. Anyone may be get cryptosporidiosis but it most commonly affects children under the age of 10 years. The present study aimed to determine the molecular identity and risk factors of Cryptosporidium infection in children under 10 years, referred to the health care centers of Hamadan district. This study was carried out in 2013 on 420 children (222 boys and 198 girls), referred to urban and rural health care centers in Hamadan district. Stool samples were concentrated using formalin-ether method and microscopically examined after staining with modified Ziehl-Neelsen technique. DNA was extracted using RTP Mycobacteria Kit and nested-PCR protocol amplified a 850 bp fragment of SSU-rRNA gene specific for Cryptosporidium. Of the 420 children studied, only 2 individuals (0.47%) (a 6-month-old boy and a 6-year-old girl) were found to be infected with Cryptosporidium spp. The SSU-rRNA gene fragment was successfully amplified from both positive samples. Both patients were from rural areas and have had direct contact with the livestock. The results of this study showed a presence of *Cryptos*poridium, although with low prevalence, in rural children with contact to livestock. Therefore, education system should pay more attention to health knowledge improvement, especially in villages and mothers should be more educated by health care centers. Molecular characterization on human and animals isolates is needed to be done to increase our knowledge about Cryptosporidium transmission routes and its epidemiology.

Keywords: children, Cryptosporidium, SSU-rDNA, Hamadan.





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SURVEY OF SARCOCYSTIS INFECTION IN SLAUGHTERED CATTLE AND SHEEP BY NAKED EYE INSPECTION AND DIGESTION METHOD IN HAMADAN SLAUGHTERHOUSE, IRAN IN 2014

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The protozoan parasite of Sarcocystis is one of the most commonly found parasities in domestic animals worldwide. Some species of Sarcocystis can cause important economic loss when producing clinical and subclinical disease. The aim of this study was to determine the prevalence of Sarcocystis in slaughtered cattle and sheep in Hamadan slaughter house by macroscopy and pepsin digestion methods. The prevalence of Sarcocystis spp. infection was investigated in 324 cattle and 334 sheep, slaughtered from June to August 2014 in Hamadan slaughterhouse, using naked eye inspection for macroscopic Sarcocysts, and pepsin digestion, muscle squash, squeezing methods for microscopic types of sarcocysts. Muscles from heart, tongue, diaphragm, thigh, esophagus costal muscles were examined for Sarcocystis cysts. No macroscopic cyst was found in examined cattle carcasses but, the prevalence of microscopic Sarcocystis cysts in cattle was detected in 100% of carcasses. The prevalence of Sarcocystis infection in the sheep was 100% by digestion method and microscopic examination, however, about 49.3% and 34.5% infection rates were found in the diaphragm and esophagus muscles, respectively. The pepsin digestion method is found the most sensitive method for diagnosis of Sarcocystis in the meats of animals. Although 100% of cattle and sheep were found infected with Sarcocystis but the majority of the cysts were demonstrated microscopically. This means, the meat should be cooked sufficiently without considering the macroscopically meat inspection results.

COMPARISON OF FECAL ANTIGEN DETECTION WITH AURAMINE-PHENOL STAINING METHOD FOR DIAGNOSIS OF HUMAN CRYPTOSPORIDIO-SIS

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Cryptosporidium is a coccidian parasite causes cryptosporidiosis in human, which is characterized by diarrhea. Fecal antigen detection using ELISA is claimed to be sensitive than other conventional methods for diagnosis of cryptosporidiosis. The aim of the present study was to evaluate fecal antigendetection and auramine-phenol staining methods for diagnosis of human cryptosporidiosis. A total of 228 fecal samples were collected from residents of rural areas of Hamadan, west of Iran. Each sample divided into two parts, one kept frozen at -20°C for Ag-capture ELISA and the other preserved in 10% formalin for auramine-phenol staining method. Qualitative Cryptosporidium Ag-detection ELISA (CY-PRESS DIAGNOSTICS, Belgium) was performed according to manual of the manufacturer. The preserved samples concentrated using formalin-ether concentration technique stained with auramine-phenol stain and then studied under florescent microscope. Eight (3.5%) and three (1.3%) out of 228 fecal samples were positive for Cryptosporidium infection by ELISA and AP staining methods, respectively. Cryptosporidium Ag-detection using ELISA showed higher frequency of the infection compared to auramine-phenol staining method (P=0.062).For epidemiological studies and diagnostic purposes of Cryptosporidium infection especially in asymptomatic individuals, Ag-detection ELISA is an easy to perform with higher sensitivity compared to other conventional microscopic methods.

Keywords: Cryptosporidium, ELISA, auramine-phenol, antigen detection

Keywords: Sarcocystis, cattle, sheep, Hamadan





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*Guilan, Iran; 20-22 May 2015* MOLECULAR SURVEY ON PREVALENCE OF SUBTYPI

## CRYPTOSPORIDIUM AMONG DOGS OF AHWAZ

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Cryptosporidiosis is a parasidic disease caused by a parasite from the order of Coccidia called Cryptosporidium. This unicellular parasite causes diarrhea in human and domestic animals such as Dogs, cattle, Sheep and Poultry. So, because of the Zoonotic role and worldwide distribution of this parasite, evaluationg the prevalence rate of Cryptosporidium in humans and animals is of great importance in term of disease control. The aim of this study was to determine the prevalence of Cryptosporidium in dogs of Ahvaz. In this study, 200 excrement samples from 28 villages in the suburbs of Ahvaz (west, north, east and south) were collected. After condensation of the samples through Sheether's method and preparation of thin sheets, improved Zeel - Nelson staining was performed and the positive samples were marked. Then DNA was extracted from the samples by Bioneer kit. Next using 2 polymers designed based on locus ssurRNA, one piece of DNA including 720 bp of nucleotides were reproduced through PCR method. The product of PCR was put on agar gel 2% and stained by etidium bromide and then electrophoresized. In this study 15 samples (7.5%) out 200 samples were positive through staining. All the samples were investigated with molecular method and 29 samples (14.5%) were positive through PCR. In the western villages of Ahvaz 11 positive cases (5.5%) were observed and in the other areas the infection was dispersed. The present study confirms the prevalence of Cryptosporidium among dogs of Ahvaz and indicates dogs as one of the sources of Cryptosporidium which can infect humans and animals. Therefore, hygienic measures should be considered when being in contact with the dogs.

Keywords: Cryptosporidium, dog, PCR, Ahvaz

SUBTYPING OF CRYPTOSPORIDIUM SPP. IN DI-ARRHEIC CHILDREN FROM GONBAD KAVOOS, IRAN

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Cryptosporidium is an intestinal protozean parasite causing waterborne and foodborne outbreaks of diarrheal diseases. The present study was carried out in order to subtyping Cryptosporidium isolates among diarrheic children in Gonbad Kavoos city, Golestan province, northern Iran. Fecal samples were collected from diarrheic children. The primary parasitological tests was made based on detection of oocysts employing the modified Ziehl-Neelsen acid-fast staining method. The positive microscopically samples were chosen for sequence analysis of partial 60 kDa glycoprotein (gp60) gene.Out of 547 collected diarrheic samples, 27 (4.94 %) were positive for Cryptosporidium oocysts. Sequences analysis of gp60 gene in 15 Cryptosporidium isolates revealed C. parvum in all of them (100%). The results indicated three subtypes of IIa subtype family (7 cases) including IIaA16G2R1, IIaA17G1R1, IIaA22G3R1 and one subtype of IId subtype family (8 cases). The most prevalent allele was IId A17G1d (53.3%). The predominance of zoonotic subtype families of C. parvum species (IIa, IId) in the present study is in agreement with previous studies in Iran and confirms the importance of zoonotic transmission of cryptosporidiosis in the country.

Keywords: Cryptosporidium, children, subtypes, gp60 gene





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## MOLECULAR IDENTIFICATION OF SARCOCYST-IS SPECIES OF SHEEP IN BABOL, IRAN

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To differentiate Sarcocystis macro-cyst forming species in slaughtered sheep in northern Iran, PCR and sequence analysis of the 18S rRNA gene was performed. Also, autofluorescence ability of bradyzoites isolated from fifty macro-cysts was assessed. One-hundred fifty carcasses of sheep were checked macroscopically to find macro-cysts infection of Sarcocystis species. One macro-cyst was isolated from the infected oblique abdominal and diaphragm muscles and used for microscopic and molecular studies. PCR of the 18S rRNA gene was used for molecular analysis. A smear was prepared from washed fresh zoites obtained from each macro-cyst to perform microscopic examination such as autofluorescence. The prevalence rate of Sarcocystis infection was 33.3% (50 / 150). All bradyzoites isolated from the macrocysts glowed green when examined with an ultraviolet microscope. The partial 18S rRNA gene of Sarcocystis species was amplified at the expected PCR product size (~1100 bp) from all samples. Overall, twenty samples (66.7%), six (20%) and four (13.3%) isolates were identified as S. gigantea, S. moulei and Sarcocystis spp. by BLAST analysis, respectively. This work revealed that sheep is an alternative intermediate host for S. moulei. To better understand the genetic diversity among Sarcocystis species, complete sequences of the 18S rRNA gene or sequence analysis of other genetic loci are recommended.

Keywords: Sarcocystis, PCR, 18S rRNA, S. gigantea, S. moulei

SOIL CONTAMINATION WITH CRYPTOSPORID-IUM SPP. IN KERMANSHAH, WESTERN IRAN

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Cryptosporidium is a coccidian protozoan parasite that causes gastrointestinal disorders in human and animals. Soil is an important source for disease transmission. The aim of this study was to find Cryptosporidium spp. oocysts in the soil collected from primary schools and parks in Kermanshah city. This survey was conducted from August to December 2014 in Kermanshah. Altogether, 192 randomly selected soil samples were collected from 24 parks and 24 primary schools in 6 regions. Cryptosporidium oocysts were isolated using flotation technique, stained with cold modified Ziehl-Neelsen procedure, and examined by a light microscope for the presence of Cryptosporidium oocysts. 49 (25.5%) out of 192 soil samples were found to contain Cryptosporidium oocysts. The contamination rate in public parks and elementary schools were observed 21.9% and 29.2%, respectively. Data analysis by chisquare test revealed no significant relationship among parks and primary schools in terms of the contamination rate ( $x^2 =$ 1.34 andp=0.24). Furthermore, there was no significant difference between the contamination rate and different regions of Kermanshah (x2= 5.39 and p=0.36). The regions 3 and 4 by 34.4% had the highest contamination rate and the lowest was for the region of 6 with 15.6% contamination rate. Considering human infection with different Cryptosporidium species and an increase in immunocompromised patients, high contamination soil related to this parasite in Kermanshah is a serious problem. Consequently, health promotions, public education, improving sanitation conditions, especially in underprivileged areas, are the keys to success in preventing the spread of Cryptosporidium infection. In this regard, findings of this study can be used as a basis for preventive programs and strategies targeting those groups who are at the greater risk of cryptosporidiosis.

Keywords: Cryptosporidium, Kermanshah, primary schools, soil





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IDENTIFICATION OF CRYPTOSPORIDIUM SPP. IN HOUSEHOLD DRINKING WATER REFINERY SYSTEM IN KHORRAMSHAHR CITY, SOUTH-WEST IRAN

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Water is the most important necessity in creatures' survival. For this case, preventing water pollution is equally important. According to CDC, various microorganisms including Cryptosporidium spp. can be transmitted by different sources of water and infect human beings and animals. The aim of this research was to study the existence of Cryptosporidium oocyst in drinking water of Khoramshahr city, before, after and in filters of household refinery system by Sheather's method, modified Ziehl Neelsen staining and Nested- PCR. Twenty samples were collected randomly from four districts of the city (5 samples of each district, North, South, East and West). The samples were collected by referring to the houses using refinery filters. Three samples were collected from each filter, one liter of pre- filtration and one liter post- filtration and then the old filter was removed and new filter was installed. The samples of the water and filters were used for examination. Totally 60 samples were collected from 20 houses. The samples were examined using Sheather method, modified Ziehl Neelsen staining and Nested- PCR. In 2 out 20 precipitate from the filters were positive for Cryptosporidium spp. only by PCR. In none of the pre and post filtered water Cryptosporidium spp. was detected by Sheather and Modified Ziehl Neelsen staining methods. The results of this study indicated that Cryptosporidium spp. is present in 10% of drinking water of Khoramshahr city, and it is suggested that all household should use water refinery system and obtain safe drinking water.

Keywords: Cryptosporidium, water filtration, drinking water, Iran

## DETECTION OF CYCLOSPORA CAYETANENSIS IN A HIV POSITIVE PATIENT WITH DIARRHEA FROM TEHRAN: A CASE REPORT

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Coccidian protozoa of Cyclospora cayetanensis is an obligatory intracellular Apicomplexan parasite which infect the mucosal epithelium of the small intestine of immunocompetent and immunocompromised individuals. Clinical laboratories are not very much experienced for diagnosing Cyclospora oocysts. It is important to distinguish the oocysts of Cyclospora from those of Cryptosporidium. A 25- years old woman from Tehran surroundings with complaint of faintness and fatigue with HIV positive confirmed eight months ago admitted in Imam Khomeini hospital .The patient suffered from intestinal and lung symptoms like watery diarrhea and flu-like symptoms. The stool sample referred to laboratory of intestinal protozoology at the School of Public Health, Tehran University of Medical Sciences and was examined by direct preparation and concentration techniques and stained with modified acid fast staining method and observed with light and then Immunofluorescence microscope. The stool cultivation was made in potassium dichromat medium. Bilayer wall oocysts were observed in wet mount. The oocyst size was 10 µm, after 1 week oocysts sporulation the oocyst had two sporocysts. Diagnosis of Cyclospora infection was finally made according to observation of Cyclospora oocysts in acid fast staining method and autofluorescence of Cyclospora under Immunofluorescence microscope. The patient was initially treated with azithromycin, tazocin and fluconazol because of lung lesions and diarrhea and relative remission was observed. Cyclospora sp. causes an intestinal infection particularly in immunocompromised patients.

Keywords: Cyclospora cayetanensis, HIV, diarrhea, coccidian protozoa





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PREVALENCE OF INTESTINAL COCCIDIAL IN-FECTIONS AMONG DIFFERENT GROUPS OF IMMUNOCOMPROMISED HOSTS IN TEHRAN DURING 2013-2014

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Coccidian parasites are very important in human as emerging pathogens in immunocompromised individuals. Cryptosporidium and Isospora are recognized as significant and widespread causes of diarrheal illness in both immunocompetent and immunocompromised hosts. Incidence of enteropathogens such as Cryptosporidium spp. and Isospora belli considerably has increased, since immunodeficiency virus (HIV) rapidly disseminates. Also cancer patients are highly susceptible to opportunistic infections. This study aimed to estimate the prevalence of cryptosporidiosis and isosporiasis in immunocompromised patients in Tehran. This cross-sectional study carried out among different hospitals in Tehran during 2013-2014. Stool samples collected from 350 immunocompromised patients consist of 80 (23%) patients infected HIV (AIDS), 15 (4%) organ transplant recipients, 234 (67%) patients suffering from malignancy and 21 (6%) patients with other immunodeficiency diseases. All samples were suspended in PBS (PH 7.5), then filtered. Final suspension washed three times by PBS and smears prepared from sediments. Prepared smears stained by Ziehl-Nielsen technique and examined by microscopic method. Out of 350 patients, 195 (55.7%) were males and 155 (44.3%) were females. Cryptosporidium oocysts were detected in 3 (0.9%) samples consisted of one sample from HIV+/AIDS patients and 2 samples from organ transplant recipients. Isospora oocysts were detected in 4 (1.1%) samples consisted of 2 samples from HIV+/AIDS patients, one sample from patients suffering from malignancy and one sample from patients with other immunodeficiency diseases. Because of low infective dose and resistance of Cryptosporidium to conventional water disinfectants and no definitive treatment, it seems that prevention is the main effective method to reduce coccidiosis infection especially among immunocompromised individuals.

Keywords: cryptosporidiosis, isosporiasis, immunocompromised, Tehran

## FREQUENCY OF PARASITIC GASTROENTERITIS IN HOSPITALIZED CHILDREN AT PEDIATRIC HOSPITAL (HEFDAHE SHAHRIVAR, RASHT, IRAN), DURING A SIX MONTH PERIOD IN 2014

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Parasites are causes of a portion of diarrhea and gastroenteritis cases particularly in children. Major parasitic agents of gasteroenteritis are Giardia lamblia, Entamoeba histolytica and Cryptosporidium spp. They can produce sever symptoms and even hospitalize the infected children. This study was aimed to monitor the frequency of parasitic cases of gastroenteritis. This descriptive-cross sectional study carried out through searching the results of parasitic examinations in medical records of all gastroenteritis cases admitted as inpatients at pediatric hospital, Hefdahe Shahrivar, during a six month period from April to October 2014. A total of 177 patients with diagnosis of gastroenteritis and diarrhea were identified. Medical records of all cases were investigated. Parasitic examination was requested for all patients and the reported results were negative for all cases. It means that parasites comparing to other agents of gastroenteritis, eg, viruses and bacteria, were less prevalent.Findings of this study revealed that parasites were not identified in any gastroenteritis patients in the study population. However, as the proper identification of the infectious agents is the first step towards a successful treatment, hence, implementing the appropriate laboratory methods and following the principles of parasitology examinations such as performing three repeats on alternate days can help to rule out parasitic disease with confidence. Analyzing the fresh stool specimen for all diarrhetic cases and application of specific test for diagnosis of cryptosporidiosis through special concentration technique followed by Ziehl-Neelsen staining can improve the accuracy of detection of parasitic infections in patients suffering gastroenteritis.

Keywords: children, gastroenteritis, parasite, Iran.





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## SEPARATION OF SARCOCYSTIS TISSUE CYSTS FROM CALVE SKELETAL MUSCLES

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Sarcocystis spp. is a coccidian parasite and has been considered as a causal agent of an important food-borne parasitic disease in both humans and animals. This study conducted to isolate tissue cyst-forming parasites from commercial meat samples. Five grams of the meat sample obtained from calves was sliced into 2-3 sections in 30 ml of phosphate-buffered saline containing 0.1% Tween 80 and homogenized using IKA T25 homogenizer. The homogenated sample was filtrated twice and the filtrated suspension was used for microscopic examination and molecular analysis. Polymerase chain reaction (PCR) of the 18S rRNA gene using specific primers for Sarcocystis Genus and partial sequence analysis were employed to identify the parasite species. Giemsa staining of the filtrated samples demonstrated the ellipse to around tissue cysts contained crescent-shaped bodies in the sample. The PCR of the 18S rRNA yielded an 1100 bp DNA band on agarose gel and sequence analysis of the DNA established that the isolate was S. cruzi. The described method in the present work would be practical in separation of tissue cysts of coccidian parasites from meat sample in order to do further evaluation of the cyst-forming parasites.

Keywords: coccidian parasites, Sarcocystis, tissue cyst

INFECTION OF EIMERIA IN SMALL RUMI-NANT'S IN SOUTH OF IRAN

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Coccidiosis, one of the most economically important diseases of small ruminants, is caused by Eimeria spp. This parasite causes pathological damages and mortality in poultry, cattle, sheep, goats, pig, rabbit and other animals. This study was done to survey the prevalence of Eimeria in sheep and goats in Shiraz city, Iran. Fecal samples were taken directly from the rectum of 40 sheep and goats. After transferring the samples to laboratory, the Clayton lain technique was used to float and watch the oocysts of each sample. Morphological identification of Eimeria oocysts was carried out based on shape and size of the oocysts, presence or absence of the micropyle and polar cap and color and thickness of oocyst walls. Overall, infection to Eimeria spp. was seen in 15 (37.5%) examined animals. Also, 6 (15%) showed severe infection.Severe infection of sheep and goats to some species of Eimeria can cause diarrhea, dehydration, reduction of productivity, weight loss and death. Since sheep and goats can be parasitized by different Eimeria species and most of them do not cause visible clinical coccidiosis, diagnosis must be combined with species identification in order to avoid economic losses. Avoidance of overcrowding, cleaning and drying the bed and separating young animals from the old are important measures for reducing the prevalence of the disease.

Keywords: Eimeria, small ruminant, Iran





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COMPARISON OF AURAMINE PHENOL AND MODIFIED ZIEHL-NEELSEN STAINING METHODS FOR DIAGNOSIS OF CRYPTOSPORID-IOSIS IN CALVES

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Cryptosporidium, a coccidian parasite, is the causal agent of cryptosporidiosis in humans and animals. The present study aimed to determine the prevalence of Cryptosporidium infection among calves in dairy farms of Isfahan area using auramine phenol and modified acid fast staining methods. In this cross sectional study, 130 fecal samples were collected from 4 dairy farms located in the Isfahan outskirt during spring 2014. The samples were concentrated using formalinether sedimentation technique. The concentrated materials were stained with two methods including auramine phenol and modified Ziehl-Neelsen staining methods. The data were analyzed by SPSS software through McNemar test. Cryptosporidium oocysts were observed in 41 (31.53%) and 43 (33.07%) out of 130 fecal samples using Ziehl-Neelsen and auramine phenol staining methods, respectively. The difference in the frequency of Cryptosporidium infection rate among the two used method was not statistically significant (P>0.5). The prevalence of cryptosporidiosis among calves in Isfahan area is considerably high and can be regarded as a health problem for the local residents. Also both auramine phenol and modified Ziehl-Neelsen staining methods are similarly efficient for diagnosis of cryptosporidiosis in calves.

Keywords: Cryptosporidium, calves, auramine phenol, Ziehl-Neelsen

## PARASITIC AGENTS IN HOUSING REFINERY FILTERS WITH EMPHASIZE ON IDENTIFICA-TION OF CRYPTOSPRIDIUM SPECIES IN KHORRAMSHAHR, SOUTHWEST IRAN

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Water pollution is one of the main global problems that needs continuous evaluation and revising in water source policies in all fields (from international waters to inland waters and wells). It has been always pointed out that water pollution is one of the death reasons throughout the world. During the past 15 years, an increasing number of waterborne outbreaks caused by Cryptosporidium and Giardia have been documented worldwide showing a trend in which protozoa and viruses are replacing bacterial pathogens as agents of primary concern in waterborne disease. In current study we evaluated the Cryptosporidium presence in housing refinery systems. We selected 20 houses randomly from 5 regions and in each house 3 samples were taken. The samples transported to the laboratory and tested by direct, sheather, ziehl Neelsen and PCR. Out of 20 samples 10% were Cryptosporidium positive, in 8 of 20 samples, either egg or larva were observed. The results showed that the sanitation and refinery system protocols are needed to be reviewed and revised. The residents should be educated and warned about parasitic disease and the governor should obligate the use of refinery systems in houses. Because of the lower size of the Cryptosporidium oocysts (4-6µ), it seems that filters should be replaced by smaller pore size.

Keywords: Cryptosporidium, housing refinery system, health, Khorramshahr





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## PREVALENCE AND EPIDEMIOLOGICAL STUDY OF CRYPTOSPODIDIOSIS IN SHEEP OF SANAN-DAJ

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Cryptosporidium is an Apicomplexan intestinal protozoa, which infects the gastrointestinal tract of domestic animals and humans and causes varying degrees of naturally occurring diarrhea and death in neonatal farm animals and infected animals, and can be a potential risk to public health. The aim of the present study was to determine the prevalence of Cryptosporidium infection in sheep in Kurdistan district, west of Iran, from March to October 2013. For this cross-sectional study, 180 fecal samples were collected randomly from 200 farms in 9 villages in Kurdistan district. The samples were transported to parasitology laboratory. All the animals in this study were 1-14 months. Fecal samples of animals were collected directly from their rectum. The fecal samples were then fixed as quickly as possible in 10% formalin for preserving until further examination. Moderately thick fecal smears were prepared and air-dried. After methanol fixation, the fecal smears were stained by modified Ziehl-Neelsen staining. The stained smears were observed under microscope with a ×400 to ×1,000 magnifications. The prevalence of Cryptosporidium in lambs in Kurdistan province were found 6.1%. The prevalence of the infection according to age groups in lambs were as follow: less than 6 months of age 18.75%, 6-12 months: 5% and more than 12 months: 2.27% (P>0.05). The prevalence of the infection according to sex groups in lambs were 1.85% in male and 7.94% in female animals (P>0.05). The presence of Cryptosporidium spp. in lambs in Kurdistan indicates that this protozoan parasite should also be considered in the etiology of lambs exhibiting diarrhea.

Keywords: Cryptosporidium, Sanandaj, sheep

## DIGESTIVE PARASITIC INFECTIONS OF SALM-ON TRUTTA FARIO AND ABRAMIS BRAMA OF VAHDAT DAM IN SANANDAJ, IRAN

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For this purpose from October to February 2014, a total of 150 fish specimens belong to Abramis brama and Salmon trutta fario species were examined. The fish were predated from arrival area, median area and beside of the Vahdat dam in Sanandaj. After the samples transported to the laboratory parasitology, biometrics features such as total length, total weight, and the number of lateral line sequines were recorded, then digestive system of fishes were taken out and contents were investigated using a 100 pore mesh. Their GI mass were filtereted, for presence of any worm.Worms were collected and stained by carmin acid method. Our results showed that Salmon trutta fario fish were not contaminated, but neoechinorhynchus rutili (Acanthocephala) were found in Abramis brama fish inhabiting in Vahdat dam. Out of 75 fish, 59 (78.6%) were contaminated to neoechinorhynchus rutili. Acanthocephala numbers and measures were reported 1 to 18 and 3-18 mm, respectively. The present study is the first survey on digestive parasitic infections of Salmon trutta fario and Abramis brama of Vahdat dam in Sanandaj, Iran.

Keywoeds: Salmon trutta, Abramis brama, parasite, Sanandaj





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MOLECULAR IDENTIFICATION OF MACRO-SCOPIC AND MICROSCOPIC CYSTS OF SARCO-CYSTIS AND GENOTYPIG THIS PARASITE IN SHEEP IN NORTH KHORASAN PROVINCE, IRAN

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Sarcocystis is an obligatory intracellular protozoan parasite which can infect humans and animals. Sheep are intermediate hosts of four Sarcocystis species: Sarcocystis tenella, Sarcocystis gigantea, Sarcocystis arieticanis, and Sarcocystis medusiformis. The purpose of this study was to perform a molecular identification of the macroscopic and microscopic cysts of Sarcocystis, and also, identification genotyping strains of this parasite in sheep of North khorasan province. In this investigation, 320 samples of liver, diaphragm, heart and muscles for genotyping and molecular identification of macroscopic and microscopic cysts of this parasite from 80 slaughtered sheep (40 males, 40 females) were prepared. The digestion method was used for bradyzoites observation in heart, liver, diaphragm and muscle samples. PCR analysis was conducted on macroscopic and microscopic cysts and also all other samples. Sequencing was performed for ten PCR products. Genotypes were identified by BLAST search and homology analysis. Macrocysts were seen in two muscle tissues. Digestion method and PCR analysis revealed positive results in all samples taken from heart, liver, diaphragm, and muscle. Genotyping of ten tissue samples proved that the genotype of macroscopic belonged to Sarcocystis gigantea and microscopic cysts to Sarcocystis tenella. Sarcocysts were seen with histological method. Digestion method showed that all samples infected with Sarcocystis bradyzoit. Also, this study proved Genotyping of Sarcocystis belonged to Sarcocystis tenella (75%), Sarcocystis moulei (8.33%), and Sarcocystis gigantea (16.66%). Microscopic cysts are more prevalent than macroscopic cysts and they can cause enormous economic losses. Moreover, Sarcocystis tenella, Sarcocystis moulei and Sarcocystis gigantea were reported for the first time in this province and Sarcocystis tenella was the most common species.

Keywords: Sarcocystis tenella, Sarcocystis gigantea, molecular analysis, genotyping.

## AN EPIDEMIOLOGIC STUDY OF CUTANEOUS LEISHMANIASIS IN GENAVEH COUNTY, BU-SHEHR PROVINCE, SOUTHERN IRAN

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Introduction and Objectives: Leishmaniasis has a diversity of clinical signs ranging from deadly visceral to self-curing dermal ulcers. Cutaneous Leishmaniasis (CL) is the most prevalent type of leishmaniasis. The etiological agent of CL is various species of the genus Leishmania . The purpose of this study was to examine the epidemiological features of CL in Genaveh County. This descriptive study was conducted during 2004-2008. A questionnaire was completed for each case, indicating age, sex, place of residence, the number and location of CL lesions and etc. Suspected active lesions were scraped with a sterile blade and the samples smeared on to glass slides, fixed with methanol, stained with Giemsa and examined under a light microscope for the presence of amastigotes. Totally, 135 cases of CL attended at health centers. There was the highest number of catching the CL in 2006 (n=54). Most cases (56.3%) occurred during winter. The most highly infected age group was 1 to 10 years (28.2%). The hands were the most affected parts of the body. About 54% of the patients had one lesion. The most cases (53.3%) occurred in rural areas. Concluion: It is important for the health authorities to take powerful strides to control CL and pull up its distribution. Meanwhile, it is significant to prepare quick treatment of the cases.

Keywords: cutaneous leishmaniasis, epidemiology, prevalence, Iran





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Evaluation Of Levels Of Interleukin-17, 23 And Gamma Interferon In Cutaneous Leishmaniasis Patients Before And After Treatment By ELISA, In Fars Province, 2013

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In this research, the production of interleukin-17, 23 and gamma interferon at peripheral blood mononuclear cells isolated of patients with cutaneous leishmaniasis before and after treatment were compared to evaluate whether these cytokines plays any role in recovery process. Twenty-three newly infected people suffering from cutaneous leishmaniasis caused by L. major participated in this study and peripheral blood mononuclear cells (PBMCs) were isolated. 1 month after treatment, the sampling repeated. Production of IL-17, 23 and IFN-  $\gamma$  in cell culture supernatant was assayed using ELIZA method. The production of IFN- x and IL-17 in patients (before treatment, healing, and non- healing) were higher than healthy controls. Moreover, production of both cytokines in healing cases was higher than non healing patients. In this study we observed lower levels of IL-23 in ptients in comparison with healthy controls. Furthermore, Non healing cases produced higher amounts of IL-23 than healing cases. It appears that the production of IFN  $\gamma$  is necessary for recovery from leishmaniasis but further studies are required to found the role of IL-17and 23 in this disease.

**Keywords:** IFN **γ**, IL-17, IL-23, PBMC, cutaneous leishmaniasis SEROPERVALENCE OF VISCERAL LEISHMANIA-SIS BY PARASITOLOGICAL, SEROLOGICAL AND MOLECULAR METHODS IN RODENTS FROM SARAB, EAST AZERBAIJAN PROVINCE, IRAN

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Visceral leishmaniasis is a parasitic infection and zoonotic disease in the countries of Mediterranea basin and Middle East and Iran. The causative agent of visceral leishmaniasis in Iran is Leishmania infantum, and dogs are its main reservoir hosts. Infections of Leishmania infantum in rodents have been previously reported. The main objective of this investigation was to screen Leishmania infection in rodents through serological and parasitological and PCR examinations in Sarab district.Overall, 100 rodents of different Genus and species were trapped alive in various parts of the Sarab area. Before killing the rodents, blood samples were collected from each of them in EDTA tubes. Then, all of the blood plasma were tested by direct agglutination test (DAT). Amongst them 1 (1 %) of the rodent was seropositive, 6 (6%) rodents had lower titers than positive titer, and 93 rodents were seronegative. The smears were prepared from spleen and liver of all animals and examined microscopically but no amastigote obtained.PCR technique also revealed no band related to the Genus Leishmania.

Keywords: visceral leishmaniasis, rodents, direct agglutination test, PCR, Sarab





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## AN IN VITRO COMPARISON OF NON-RESPONSIVE WITH RESPONSIVE ISOLATES TO GLUCANTIME IN A FOCUS OF ANTHROPONO-TIC CUTANEOUS LEISHMANIASIS

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Leishmaniasis is an important public health problem in tropical countries including Iran. Anthroponotic Cutaneous Leishmaniasis (ACL) is restricted to humans and chemotherapy is the important method to control the disease. At present, the first - line treatment for all forms of leishmaniasis is pentavalent antimony drugs, specifically meglumine antimoniate (MA). Since resistance to MA is increasing -throughout the world including Iran, this study aimed to assess any association between clinical response and laboratory findings in patients with ACL and to evaluate the susceptibility level of Leishmania tropica to MA. Thirty ACL patients consisting of 20 sensitive and 10 resistant cases were randomly selected. Writen informed consent of the patients was obtained. The level of susceptibility of promastigote and amastigote stages of isolates was evaluated in culture media by MTT assay and macrophage model, respectively. In addition, for both sensitive and resistant isolates, the IC50 values (50% inhibitory concentration) were calculated. Data were entered in a computer, using SPSS ver18 and for analyses of data mixedmodel and Pierson Probit regression test were performed (p<0.05 were considered as significant). Analysis of data showed an association between clinical response and laboratory findings and the IC50 values for the Glucantime - resistant isolates were significantly higher (235.92 µg/ml and 74.55  $\mu$ g/ml, respectively) in amastigote stage than the promastigote form (489.55 µg/ml and 190.76 µg/ml, respectively ). In other words, the IC50 values in Glucantime -resistant isolates were 3- fold higher than the corresponding sensitive ones in amastigote , while it was 2.5-fold higher in promastigote than the sensitive isolates (p < 0.001)Findings of the present study show that clinical resistant patients to Glucantime are also resistant in laboratory assays. Further investigations are needed to identify these isolates by molecular techniques. Such experiments are essential for future control strategies.

Keywords: meglumine antimoniate, *Leishmania tropica*, amastigotes, promastigotes, resistance

IDENTIFICATION OF LEISHMANIA SPECIES CAUSING CUTANEOUS LEISHMANIASIS BY PCR METHOD IN SARAKHS, IRAN DURING 1392

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Cutaneous leishmaniasis is one of the most common parasitic skin infections caused by different Leishmania species. Different parts of Iran including Sarakhs in Khorasan Razavi province are considered as endemic areas for this disease. Diagnosis of Leishmania species is not possible by microscopic techniques due to their morphological similarity. Therefore, molecular methods, especially PCR (according to the specificity of DNA) is useful to identify the species of parasite for reservoirs combating. According to previous studies Sarakhs is considered as an important focus for the L. major. Due to its location on the Silk Road and proximity with Turkmenistan due to common borders and increase in trafficking for commercial purposes, we decided to use PCR method for identification of parasite species. In this study, we extracted DNA of 80 samples from their direct slides by DNA assay mini kit (Ge Net Bio). Extracted kinetoplast DNA was amplified by PCR method using two specific primers. Electrophoresis patterns from each isolated sample were compared with reference strains of Leishmania major, Leishmania tropica and the marker. The results of kDNA gene templates in electrophoresis gel showed that 64 of 80 cases were positive for Leishmania and 12 cases belonged to L. tropica and 52 for L. major in comparison with standard patterns. The results of this study showed the existence of L. tropica in addition to L. major in Sarakhs. This could be due to its specific situation like proximity with international railroad, border with Turkmenistan and increase of transportation to other parts of the country and even other countries in last decades.

Keywords: Leishmania species, PCR, Sarakhs, DNA assay





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EPIDEMIOLOGY OF CUTANEOUS LEISHMANIA-SIS IN PATIENTS REFERRED TO THE SARAKHS CENTER, KHORASAN RAZAVI PROVINCE IN 2012-2013

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Leishmaniasis occurs in 88 tropical and subtropical countries. About 350 million people live in these areas. Leishmaniasis occurs commonly in Iran. The purpose of this study was to evaluate the epidemiological factors affecting the disease in Sarakhs area. This analytical - descriptive study was performed on 80 patients with suspected cutaneous leishmaniasis during 2012-2013 in Sarakhs health center. After clinical inspection for final diagnosis the patients were referred to laboratory where, sampling and Giemsa staining were done for Leishman bodies. Patients' demographic data such as sex, age, occupation and existence of reservoirs in their place of residence and its surroundings were collected.Leishman bodies were proved in 60 cases (75%) of 80 patients (61% male and 39% female) referred to this center. The majority of the positive cases belong to age group 10-20 years (about 31%) followed by age group 20-30 years (20%). Workers with 50% (30 cases) and students with 18% (11 cases) were the most infected occupational groups. Presence of the reservoirs in residential places were reported by 51.5% of the patients, from which 51% were gerbils and 49% dogs. According to the study: 1 - No significant difference observed between sexes and risk ratios of the both sexes were almost identical, 2- According to the higher frequency of the disease in 10-20 years age group and in workers, necessary measures for prevention should be considered for these groups, 3- Low infection in age group of 40-50 and more than 50 years can be attributed to the presence of immunity due to the previous infection 4- Removing the dogs and infected gerbils from endemic areas can play an important role in reducing disease prevalence.

Keywords: epidemiology, cutaneous leishmaniasis, Khorasan Razavi THE STUDY OF WOUNDS IN 195 PATIENTS WITH CUTANEOUS LEISHMANIASIS IN MEDI-CAL COLLEGE OF MASHHAD UNIVERSITY OF MEDICAL SCIENCES DURING 2009-2012

<u>Bibi Razieh Hosseini Farrash</u><sup>1</sup>, Ali Akbar Shamsian<sup>2</sup>, Abdolmajid Fata<sup>3</sup>, Abdorrahim Rezaee<sup>4</sup>, Mohammad Reza Hedayati Moghaddam<sup>5</sup>, Elham Poustchi<sup>6</sup>, Amin Akbarzadeh<sup>7</sup>, Fatemeh Sad Abadi<sup>8</sup>

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Cutaneous leishmaniasis is the most common form of leishmaniasis transmitted by sand fly bites. Iran, including Khorasan province, is one of the endemic areas for L. tropica and L. major. The aim of the present study was to evaluate leishmaniasis lesions in various parts of the body and the incidence of wounds in different seasons in orther to find the ways for prevention and control of the disease. This cross-sectional study has been carried out on 195 patients with cutaneous leishmaniasis diagnosed by laboratory methods during 89-92. The Frequency, average number and appearance of wounds were examined in different parts of the body and their incidence in different seasons. Then the collected data were analyzed. 41% of people had one cutaneous lesion. The appearance of lesions in 26.6% was humid and 26.6% was dry. Highest percentage of wounds was 38.3% in the hands, the legs with 24.2% and the face with 20.9%, respectively. The highest incidence of lesions in patients referred from major endemic areas was observed in October and November, with 53% and the lowest incidence observed from March until the end of spring with 6.6%. While the most incidence in endemic areas for minor species were in February and then March and April, with 53% and lowest was in August with one case. According to this study: 1 - Statistically, there isn't any significant difference in the average number and size of lesions. 2 - The highest percentage of wounds has been observed on the hands and feet, thus covering the body in endemic areas can play an important role in prevention. 3 - Like other similar studies, the highest percentage of lesions is belonging to hands and feet. 4 - The high incidence of lesions were observed in the October and November for L. major and February and March for L. tropica so according to incubation period for each species, peak activity of sand fly vectors can be considered in the summer and autumn, which necessiates the measures for combating the vectors in these seasons.

Keywords: wounds, cutaneous leishmaniasis, Mashhad





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#### EFFECT OF A NEW SYNTHETIC MATERIAL (CW) ON PROLIFERATION OF *LEISHMANIA MAJOR* PROMASTIGOTE IN CULTUR MEDIA

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The genus of Leishmania as two-host protozoa is the causative agent of leishmaniasis. One of the important choices for appropriate prevention is leishmanization. In this process, it is necessary to have multiple growths of the promastigotes in culture media. Fetal calf serum (FCS) is the main part and the most expensive ingredient of the Leishmania culture media. In this study, the effect of a new synthetic material made from cheese (CW) - comprised of most important proteins- was investigated as a substitution for FCS. Leishmania major (MRHO/IR/75/ER) stored at department of parasitology from Shahid Sadoughi University of Medical Sciences was used in this study. Different concentrations (1%, 5%, 9% and 10%) of filterd CW were evaluated as a growth stimulator in Leishmania culture procedure. This study showed that the concentration of 9% CW has the efficacy on the growth of promastigotes inside the culture media. The results of the present study introduced an alternative low-cost material made from cheese and full of necessary proteins for growth of promastigotes in culture media that could be replaced for FCS.

Keywords: Leishmnia, culture media, promastigote

LEISHMANIA INFANTUM DNA DETECTION IN PHLEBOTOMUS TOBBI IN A NEW NORTHERN FOCUS OF VISCERAL LEISHMANIASIS IN IRAN

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The human leishmaniasis is the most diverse and complex of all vector-born diseases in their ecology and epidemiology. Phlebotomin sand flies (Diptera: Psychodidae) are the sole vectors of Leishmania, and species of the genus Phlebotomus are the only known vectors in the ancient World. The main objectives in this study were to identify the vector(s), the parasite and the species composition of sand flies in Savodjbolagh district, Alborz province. This study was carried out from early May to October 2012 in 4 villages, Kordan, Agasht, Arab-Abad and Hiv in Savodjbolagh County. Sand flies were collected using sticky traps biweekly from indoors and outdoors. For reaching our objectives, we used polymerase chain reaction of kDNA, ITS1- rDNA, followed by Restriction Fragment Length Polymorphism (RFLP). Altogether 7930 sticky traps were installed and 1034 specimens comprising 8 species of sand flies were collected and identified. Two species of Phlebotomus sergenti and Phlebotomus tobbi were the most prevalent among 8 species identified comprising 51.1% and 32.9%, respectively. Among the 160 specimens of female sand flies tested by polymerase chain reaction of kDNA, ITS1- rDNA, followed by restriction fragment length polymorphisms, only 1 out of 80 Phlebotomus tobbi (1.25%) were positive for Leishmania infantum parasites. Conclusions: Finding naturally infected wild-caught sandflies and their ability to feed on humans (anthropophilic) fulfills two essential requirements for incriminating a sandfly as vector. Our finding showed that Phlebotomus tobbi might play a role as a vector in circulating Leishmania infantum between reservoir (s) and humans.

Keywords: Leishmania infantum, vector, Savodjbolagh, Alborz, Iran





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#### DETECTION OF LEISHMANIA SPECIES FROM CLINICAL SPECIMENS BY MOLECULAR METH-OD IN KASHAN, IRAN

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Leishmaniasis is one of the infectious parasitic diseases of highest incidence in the world. Cutaneous leishmaniasis is one of the health problems in Iran including Kashan province. This study aimed to identify the Leishmania species in cutaneous leishmaniasis by PCR using primers of kinetoplastic DNA for treatment and controlling of the disease. 130 patients suspected to cutaneous leishmaniosis referred to two health care centers of Kashan from 2012 to 2013 were examined. The demographic data as well as signs of the disease were recorded. The diagnosis of infection was based on identification of amastigotes within the smear of serosity of the wound, after staining by Giemsa. Then, Leishmania species was identified following the extraction of DNA from serosity using PCR with variable region of the KDNA. Expected PCR products of L. major and L. tropica were 650 bp and 760 bp, respectively. Overall of 130 specimens, 87 (66.9%) cases and 98 (75.4 %) cases were positive for microscopy and PCR methods, respectively. In PCR assay 70 (71.4 %) and 26 (26.6%) of the samples were identified as L. tropica and L. major, respectively and 2 (2 %) cases were detected as mix.Based on the results of this study, both species of Leishmania were present in Kashan. Therefore, it is suggested that careful preventive measure be taken in rural and urban parts. In addition, KDNA-PCR assay using serosity for accurate and quick diagnosis of cutaneous leishmaniasis is recommended.

Keywords: cutaneous leishmaniasis, PCR, KDNA

### EFFECT OF ACUPUNCTURE TREATMENT ON LEVEL OF IFN-Γ SERUM IN BALB/C MODEL OF LEISHMANIA MAJOR INFECTION

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Cutaneous leishmaniasis (CL) is an endemic parasitic disease in Iran. Current treatments for the disease are not satisfying, have many side effects and are expensive. The healing affect of T helper1 (Th1) immune response, especially IFN-y secretion in CL has been previously documented. It has been shown that acupuncture, a traditional Chinese medicine, also might activate Th1 immune response. In this study, the effect of acupuncture on serum level of IFN-y in experimental CL of Balb/c mice was investigated. Sixty Balb/c mice were experimentally infected with Leishmania major and assigned randomly in to three groups: acupuncture treatment group, which were given the acupuncture (thirty minutes daily, two days a week for 10 sessions), and intra peritoneal Diazepam as a sedative agent, control group for Diazepam which only received the Diazepam and a control group without any intervention. Serum level of IFN-y was measured by means of ELISA at the beginning of the study and at sessions five and 10 of acupuncture treatment in three groups. Mean serum level of IFN-y was not significantly different between and within groups at the beginning and at the sessions five and ten of the therapy. This investigation showed that acupuncture may not affect the serum level of IFN-y in Balb/c model of CL. Moreover, Diazepam does not interfere with the serum level of IFN-y in such a study. Analysis of other immune factors and early measurement of IFN-y in the course of treatment possibly may display the activated protective immune response against leishmaniasis by acupuncture.

Keywords: Leishmaniasis, acupuncture, Leishmania major, interferon- $\gamma$ 





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CONSTRUCTION OF A PLASMID ENCODING FU-SION OF LEIF-TSA ACCOMPANIED WITH GFP AS A VACCINE CANDIDATE AGAINST LEISHMANIA MAJOR

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Anti leishmanial drugs have considerable problems due to high cost, toxicity, duration of treatment, route of entry and drug resistance. Despite many medical advances, chemotherapy alone is not able to eliminate the disease. Then, making an effective vaccine for the control and elimination of the disease is essential. Leishmania promastigotes was cultivated and genomic DNA was extracted. Then, LeIF gene was amplified using specific primers; PCR product was cut with EcoRI and Hind III and cloned into pEGFP-N1 plasmid. Colony PCR and enzymatic digestion confirmed cloning. TSA gene was digested from pEGFP-TSA and subcloned into BamHI and KpnI restriction sites of pEGFP-LeIF and fusion amplification accompanied with GFP were confirmed by PCR and enzymatic digestion. The results of LeIF amplification shown a fragment about 1212bp confirming its cloning and the PCR results of LeIF -TSA fusion determined a fragment almost 1800bp and it was confirmed fusion cloning into pEGFP-N1 plasmidIn this study, cloning of LeIF-TSA fusion was correctly done and we can use it as a vaccine candidate for future studies. Since this fusion contains a gene encoding GFP, protein can evaluate the expression by fluorescent microscope.

Keywords: Leishmania major, vaccine, LeIF, TSA

DIAGNOSTIC VALUE OF MOLECULAR, MICROS-COPY AND CULTURE METHODS FOR DIAGNO-SIS OF CUTANEOUS LEISHMANIASIS

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Cutaneous leishmaniasis (CL) is an endemic disease in Isfahan province, Iran. Considering the increase in the incidence of CL in Kashan, determining the best method for diagnosis and molecular characterization of Leishmania species is necessary for effective treatment and control of the disease. This study was designed to compare microscopic, culture and PCR methods for diagnosis of cutaneous leishmaniasis. In this study, performed from 2012 to 2013, 130 patients who had referred to health care centers of Kashan as suspected cases of cutaneous leishmaniosis were examined. The demographic information as well as signs of the disease was recorded. Serosity of wound was collected for smear preparation and also cultured in Novy-Nicolle-McNeal (NNN). In addition, DNA was extracted from serosity and Leishmania species were identified by PCR by specific primers for variable region of the KDNA. PCR products of L. tropica and L. major were760 bp and 650 bp, respectively. The diagnostic criteria of cutaneous leishmaniasis were based on observation of amastigotes in the smear or promastigotes in culture or presence of expected bands in PCR. The data were analyzed by SPSS ver .16 and sensitivity and specificity and positive and negative predictive values were calculated. The data were analyzed using Chi square and Fisher's Exact test. Overall, of 130 specimens, 87 (66.9%), 72 (56.2%) and 98 (75.4 %) cases were positive for microscopy, culture and PCR, respectively. 66 (69.5%), 27 (28.4%) and 2 (2.1%) isolates were L. tropica, L. major and mix respectively. In present study sensitivity, specificity, positive and negative predictive values of PCR were 99, 100, 100 and 96.9% respectively. Based on the result of our study, kDNA-PCR assay is the most sensitive method for diagnosis of cutaneous leishmaniasis.

Keywords: cutaneous leishmaniasis, PCR, culture, microscopy, diagnosis





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STABILITY OF FREEZE-DRIED SERA STORED AT DIFFERENT TEMPERATURES FOR THE DE-TECTION OF ANTI-LEISHMANIA INFANTUM ANTIBODIES USING DIRECT AGGLUTINATION TEST

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This study aimed to evaluate freeze-dried sera as an alternative to non-freeze dried for detection of anti-Leishmania infantum antibodies over the course of 11 months using the direct agglutination test (DAT). Altogether, 60 serum samples (30 from humans and 30 from dogs) were collected from various geographical locations in Iran. All the collected sera were pooled and each pooled serum sample contained 10 different sera. In the beginning, the human and dog pooled sera were categorized as positive (weak and strong) and negative based on anti-L. infantum antibodies using the DAT. All the freezedried and non-freeze-dried sera were stored at -70°C, -20°C, 4°C, 22-28°C and 56°C for at least 330 days. The positive and negative human and dog pooled sera were separately tested using the DAT each month and the results were compared to non-freeze-dried sera kept under the same conditions. We found strong agreement (100%) between the results obtained from freeze-dried human and dog sera kept at -70°C, -20°C, 4°C and 22-28°C during this study. The human and dog pooled sera stored at 56°C were corrupted after 2 weeks. The DAT results were highly reproducible using freeze-dried human pooled sera (CV = 0.036). Conclusions: Freeze-dried human and dog sera are highly stable under different temperature conditions, are easy to transport and are safe for use as positive and negative serum controls in laboratories.

Keywords: Leishmania infantum, freeze-dried sera, preservation INCIDENCE TREND OF CUTANEOUS LEISH-MANIASIS AND MOLECULAR IDENTIFICATION OF LEISHMANIA SPP. ISOLATED FROM HUMAN IN PAKDASHT DISTRICT, TEHRAN

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Cutaneous leishmaniasis (CL) is considered as one of the most common infectious diseases in Iran. Recently, the sporadic cases of CL has been reported in some parts of the Pakdasht district located near Tehran, as old focus of anthroponotic CL (ACL). Accordingly, survey of the present situation of CL is important in this area. The aim of this study was to determine the incidence trend of CL and identification of Leishmania spp. in Pakdasht district. This cross-sectional study was performed among suspected of CL referred to health centers of Pakdasht, from March 2009 to November 2014. After registration the patient characteristics, diagnosis of disease was arranged based on direct smear of skin lesions and Giemsa staining. The kinetoplast DNA was then extracted with phenol-chloroform-Isoamyl alcohol and then species PCR method was used for the determination of species. From a total of 96 examined samples, 58 were males (60.4%) and 38 were females (39.6 %). Of these the results of direct smears were positive for 58 subjects, 31 males (55.4%) and 26 females (45.6 %). The highest incidence rate of CL (n= 31) belonged to October and November throughout 2012. The most highly infected patients were aged <10 years old (30.2 %) and the least 10 - 20 years old (13.6 %). The majority of the patients had a history of travel to Sabzevar district as an endemic area in Khorasan Razavi province and also most of patients were labourer. In this investigation the species of Leishmania parasites isolated from all samples with species -specific PCR method was L. major. Based on the results, it seems that the Pakdasht district is not considered as a cutaneous leishmaniasis focus and it has been occurred as a result of the travelling to the main endemic foci of CL.

Keywords: cutaneous leishmaniasis, PCR, Pakdasht





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## TRI-GENE FUSION OF LEIF-LACK-TSA (LLT) OF LEISHMANIA MAJOR INTO PLEXSY-NEO2 VEC-TOR AS A NEW VACCINE CANDIDATE

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A number of antigens have been identified as protective against leishmaniasis but a single antigen may not efficiently protect the human population with different immunological backgrounds. Thus, it is important to use several protective antigens. In the study, we fused three genes of L. major into pLEXSY- neo2 vector as a vaccine candidate. N-terminal part of LeIF gene was amplified by PCR from genomic DNA and was cut with Hind III and EcoRI enzymes then it was cloned into recombinant pEGFP-N1- LACK-TSA plasmid upstream of the GFP gene. The integrity of cloning was carried out by Colony PCR with forward LeIF and reverse TSA primers. Then, the LeIF-LACK-TSA (LLT) - EGFP Fusion was digested with BglII and NotI and subcloned into pLEXSY-neo2 vector at the same sites and the correct insert orientation was confirmed by enzymatic digestion. Amplification of LeIF gene was correctly done and revealed a fragment about 408bp. Moreover, the result of the colony PCR confirmed LeIF cloning into recombinant pEGFP-N1- LACK -TSA plasmid and showed a fusion fragment about 1941bp. Enzymatic analysis of pLEXSY-L.L.T.GFP confirmed the cloning correctly and showed a fragment about 2600bp. The previous studies reported that use of several antigenic components was more protective than a single antigen.We constructed a plasmid encoding tri-gene fusion of Leishmania to transfect into L. tarentolae and we can use a recombinant parasite against leishmaniasis while it cannot cause disease in human, therefore, it may be a suitable candidate for development of an effective vaccine against leishmaniasis.

HIGH OCCURRENCE OF SARCOCYSTIS CYSTS IN MEAT PRODUCED IN YAZD, CENTRAL IRAN

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Sarcocystosis is one of the most widespread parasitic diseases in cattle caused by three main species of Sarcocystis genus. The major aim of this study was to determine the prevalence of Sarcocystis spp. in meat produced in Yazd, central Iran with special reference to species identification, using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique. From March 2012 to May 2013, samples were randomly collected from esophagus, heart, diaphragm, intercostal muscle and tongue of 120 slaughtered cattle in Yazd, central Iran. After DNA extraction, PCR-RFLP was used for detection and identification of Sarcocystis spp. The statistical analysis was performed by Chi-Square test, using SPSS software (v. 16.0). The molecular analysis showed that Sarcocystis spp. was found in 112 of 120 (93.3%) slaughtered cattle. The prevalence of S. cruzi, S. hirsuta and S. hominis were 90%, 38.3% and 57.5%, respectively. Among the 112 infected cattle, single and multiple (infected by more than one species) infection were seen in 45 and 67 cattle, respectively. No significant association was detected between sex, age, sample type and the prevalence of Sarcocystis spp. (p>0.05).Considering its public health importance, high prevalence of S. hominis should be highlighted in this region. To the best of our knowledge, this study is the first of its kind to be conducted in Iran. Studies that are more detailed are needed to describe the distribution pattern and species identification of Sarcocycstis spp. in other regions of Iran.

Keywords: sarcosistosis, Sarcocystis, Yazd

Keywords: Leishmania, LeIF, LACK, TSA, vaccine





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DIRECT DIAGNOSIS AND IDENTIFICATION OF CUTANEOUS LEISHMANIA INFECTION ON FALSE NEGATIVE STAINED SMEARS: IN COM-PARISON WITH PCR AND MICROCULTURE METHODS

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The routine diagnosis of cutaneous leishmaniasis (CL) depends on examination of skin lesions using smears. Microscopic examination usually has low sensitivity and cannot identify even some confirmed CL cases. The aim of this study was to detect and identify Leishmania species among suspected CL cases having negative direct smears, by PCR and microculture methods. This study was carried out during 2011-2013. Among patients with suspicious skin lesions to CL referred to health center laboratories of Gonbad-e-Qabus and Torbat-e-Jam districts samples were obtained through skin scrapping and negative Giemsa stained smears were prepared. Then improved microculture (IMC) and polymerase chain reaction (PCR) on obtained samples were performed. The positive rates from a total of 131 subjects having negative smears were 25.9% (n=34), and 18.3% (n=24) by PCR and microculture methods, respectively. The highest degree of agreement was found between PCR and IMC (Kappa= 0.82, agreement=0.96%). Moreover, among the DNA extracted from smears, L. tropica and L. major were identified on 11 (32.3%) and 23(67.6%) of the smears, respectively. This study revealed PCR and microculture techniques are along with the advantages of early diagnosis of false negative CL cases. Also, we suggest the IMC as a valuable alternative diagnostic method for PCR in diagnosis of CL, particularly in endemic regions.

Keywords: cutaneous leishmaniasis, direct smear, PCR, microculture

## GEOGRAPHICAL INFORMATION SYSTEMS (GIS) BASED STUDY OF DISTRIBUTION OF CUTANE-OUS LEISHMANIASIS (CL) PATIENTS IN KOHGYLOYEH AND BOYERAHMAD PROVINCE AS A NEW EMERGING FOCUS OF CL IN IRAN

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Iran is one of the 10 countries in which 90% of human CL cases is occurred. CL is endemic in more than half of Iran provinces. Notable number of CL cases has been recorded in Kohkyloyeh and Boyerahmad province in recent years but no obvious outlook from CL emerging in this region of Iran is depicted. Our study clarified the distribution of CL cases in different counties and rural districts of this province in recent five years using GIS approach.Residential data from clinical and para-clinical confirmed CL patients were obtained from the province health centers from 2009 to 2013. The accuracy of residential data was checked by making random phone calls with some patients. Overall, 275 CL cases, with reliable data were confirmed. Geographical longitude and latitude of residential point was added to the GIS based counties and rural districts layers and all analysis objects carried out by ESRI- ArcMap 9.3 software from ArcGis softwares package (copyright 1999-2008 ESRI Inc). Counties with the most infected points were KohKyloveh (n=44), Gachsaran (n=16) and Boyerahmad (n=14), respectively. The most cases were from KohKyloyeh (n=105), Gachsaran (n=56), Boyerahmad (n=41) counties while Bahmaie (n=24), Lendeh (n=19) Choram (n=16), Dena (n=11) and Basht (n=3) counties were reported to have lower number of cases, respectively. Of 275 patients 218 were from only 13 villages out of 44 rural districts (RD) of the province including 11 RD in northwest, west and south regions. The most cases respectively registered from following points: Dogonbadan, Dehdasht, Yasuj, Lendeh, Kelayeh Sofla, Dishmook, Likak, Choram and Gorabdishmook. 160 cases were from rural points and 115 cases from cities. Conclusions:Most cases were from presumed west-south band while the least cases were from a north-northeast band. Regions with the most cases were in border of Khuzestan province in west and also Fars province in south regions and so Kohgyloyeh and Boyerahmad Leishmania cases, were probably originated from the endemic area of these provinces. Although northeast region is in front of Isfahan province, Dena Mountains separate these neighbourhood area and decreases related bio-relationships. Furthermore, very cold weather condition in north and northeast regions may decrease CL frequency. This study reveals precise evidences about existence and trend of distribution of CL in this province which can guide researchers for the control of CL disease.

Keywords: cutaneous leishmaniasis, Kohgyloyeh and Boyerahmad, GIS





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## CUTANEOUS LEISHMANIASIS BURDEN AND IMMUNOHISTOCHEMICAL ANALYSIS IN RE-SISTANT PATIENTS IN KERMAN PROVINCE

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The province of Kerman is one of the main focus for cutaneous leishmaniasis (CL) in the country. In recent years, Glucantime failure is raised as a health problem in treatment of CL. In various studies, immunohistochemistry of inflammatory cells is carried out in skin lesions of various species of Leishmania but no study on immunohistochemistry and phenotype of inflammatory cells of skin lesion with Leishmania tropica has been performed in patients with treatment failure. This study aimed to determine the parasite load, phenotype and immunohistochemistry of inflammatory cells in the cutaneous lesions of L. tropica among the patients with treatment failure. Mterials & Methods: Biopsy punch specimens were taken from the lesions of 35 patients consisting of 30 cases of unresponsive and 5 cases of sensitive to Glucantime. The demographic and clinical features of patients including age, gender, and location and number of lesions, and also type of treatment were recorded. After preparing paraffin blocks of samples, the immunohistochemistry tests, Hematoxylin and Eosin staining (H&E) and molecular identification of cases were performed. The data were analyzed by SPSS software ver19 using Pearson Correlation, chi-square, t-test and ANOVA. P<0.05 was considered significant. Of 30 patients with treatment failure, 63.3% were mail and 36.7% female. The mean age of male patients was 34 years and female patients were 47. Their lesions were mostly located on hands (43.3%) and face (30.1%). There was no significant difference between parasite load and gender or lesion site. There was no infected macrophage in 23.3% of treatment failured patients. The mean number of amastigotes inside infected macrophages was 11.36 and 7.4 among the non-healing and sensitive patients, respectively. The percentage of eosinophils, neutrophils and plasma cells among the patients with treatment failure was significantly higher than the sensitive cases. The mean of histiocytes in the group with low parasite load (1+) was equal to 42.2%, which was significantly lower than the mean of histiocytes (52.3%) with high parasite load (3+). There is a significant association between parasite load and dermal macrophages (CD68). This relation is positive and by increasing parasite load, the percent of CD68 cells is increased. There is a significant difference between parasite load and dermal lymphocytes (CD3). As the parasite load increased from zero to 4+, the percentages of CD3 in dermal areas is reduced and this reduction is not linear. There is no significant difference between parasite load and Langerhans cells (CD1a).It seems that in case of treatment failure, drug can exerts its control effect and avoids parasite proliferation, as only 3% of treatment failure samples have parasite loadof 4+ and can reduce infected macrophages, since 53.3% of samples had 10% or less than 10% infected macrophages. The high number of plasma cells and antibody secretion among the patients with treatment failure compared to drug-sensitive patients can indicate chronic state of leishmaniasis and development of resistance.

Keywords: cutaneous leishmaniasis, immunohistochemistry, treatment, Iran

SEROEPIDEMIOLOGICAL STUDY OF KALA-AZAR IN CHILDREN YOUNGER THAN 10 YEARS IN RURAL AREAS OF ALBORZ PROVINCE OF IRAN IN 2013

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Kala-azar is one of the highly fatal disease without treatment, especially in children younger than 10 years. The main objective of the current study was to determine the seroprevalence of Kala-azar in children under 10 y in rural regions of Alborz province. A cross-sectional study was conducted in 2013 and a multi stage randomized cluster sampling method was used for the sample collection. 50 cluster from Savojbolagh (29 cluster), Karaj (14 cluster) and Nazarabad (7 cluster) were selected. In this study 1000 blood samples were taken in heparinized capillary tubes from children under 10 y.The collected blood samples were centrifuged at 10000 xg for 5 minute and the separated plasma samples were stored at -70°C. The cut-off titers were defined as  $\geq$  1:800 for VL infection and at the titers  $\geq 1:3200$  with scientific clinical signs and symptoms as Kala-azar disease. Of the 1000 prepared plasma samples among 4 rural area, 37 (3.7%) indicated anti-Leishmania antibodies at titers ≥1:800. In Karaj rural area including Eshtehard and Maahdasht no positive serum were observed. The highest proportion of sero-positivity was in rural area of Savojbolagh. Altogether, 18 (48.6%) out of 37 of the positive serum cases were male and 19 (51.4%) were female. In this study, two children with sero-positive antibody at titers ≥1:3200 with clinical signs & symptoms including fever, splenomegaly and positive bone culture were found that were hospitalized. The findings of our study indicated that VL infection is endemic in the rural areas of Alborz province. Furthermore, it is necessary to increase the awareness of people and tourists, particularly in high-risk rural parts of the province. It seems that infected dogs and the presence of various Phlebotomus species as the vectors of the infection in these areas have provided appropriate conditions for leishmaniasis transmission to humans.

Keywords: visceral leishmaniasis, kala-azar, direct aglotination test, Alborz





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ANTI LEISHMANIAL ACTIVITY OF (2E, 5E)-5-((5-NITROFURAN-2-YL) METHYLENE)-2-(4-PHENYLTHIAZOL-2-YLIMINNO) THIAZOLIDIN-4-ONE AGAINST LEISHMANIA MAJOR (MRHO/IR/75/ER) PROMASTIGOTES IN CUL-TURE MEDIA

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Leishmania parasite is transmitted by the bite of Phlebotomus spp. which causes cutaneous, mucocutaneous and visceral leishmaniasis. The first line of leishmaniasis treatment is antimonies compounds that have side effects. In addition, there are documents that some patients showed either resistance or no response to these drugs. Therefore, a new drug is necessary to investigate appropriate effect against this important disease. In this study, effect of the mentioned drugs assessed on Leishmania major promastigote in culture media. Leishmania major (MRHO/IR/75/ER) promastigote stored at the department of Parasitology at Shahid Sadoughi University of Medical Sciences was used in this study. After abundant growth of the promastigote inside the culture media, they diluted to reach 106 promastigote per ml. Each tube with one ml solution with promastigote was exposed to the drug component with formula C17H10N4S2. The effects were assessed using means of a colorimetric assay (MTT). This study indicated that the mentioned compound in end concentration of 100 µM led to the death of 70% of the parasites. This study indicated that the mentioned compound could be as a candidate for designing an appropriate drug against leishmaniasis although more in vivo studies for finding the effects and mechanisms of the mentioned drug is necesary.

Keywords: Leishmania major, culture media, treatment

#### EPIDEMIOLOGICAL SURVEY OF VISCERAL LEISHMANIASIS IN EAST AZARBAIJAN PROV-INCE USING DAT METHOD

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Introdction & Objectives: Visceral leishmaniasis is endemic in East Azarbaijan province. This investigation was conducted in order to get some information about the prevalence of visceral leishmaniasis in East Azarbaijan province. In this descriptive (cross-sectional) study all suspected individuals during 1994-2013 were tested by serological method of Direct Agglutination Test (DAT). All of the positive cases in this survey had anti-Leishmania antibody titer equal to or higher than 1/3200 and all had symptoms and signs of the disease. This survey showed that from 1994 to 2013, the number of cases was 9, 32, 16, 75, 42, 43, 51, 40, 45, 46, 25, 17, 27, 11, 20, 21, 6, 6, 19 and 7. From 2009, the number of positive cases in male group has increased. No cases of death because of leishmaniasis has been reported. Since the disease is dorment and if it is not diagnosed on time, the rate of death will increase, initial screening using DAT test is recommended for children below 12 years.

Keywords: visceral leishmaniasis, East Azarbaijan, epidemiology





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#### EFFECT OF ARTEMISIA ABSINTHIUM EXTRACT ON THE GROWTH OF LEISHMANIA MAJOR IN BALB/C MICE

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Leishmaniasis is a diseases caused by protozoa of the genus Leishmania which is widely distributed in different regions of the world and comprises of a spectrum from a simple lesion to mucocutaneous, dessiminated and visceral diseases. Leishmania major is a causative agent of cutaneous leishmaniasis reported from around the world and Iran. Basically, variety in Leishmania species, presence of different clinical symptoms and broad dissemination of the infection has complicated the treatment of leishmaniasis. Treatment is mainly done by ntimoniate compounds, which has its own limitations and side effects. Hence, the need for more accessible, effective drugs with fewer side effects is necessary. The effect of wormwood, Artemisia absinthium - a medicinal plant native to Iran- on Leishmania major growth in macrophages of Balb/c mice were studied. Iranian species (MRHO/IR/75/ER) from Urmia Medical Faculty removed from the nitrogen tank, melted, reached to stationary phase, and injected to Balb/c mice peritoneal macrophages. Three days after injection of RPMI xtract 3% thioglycolate and Artemisia absinthium extract concentrations 800 to 25.6 mg/ml in DMSO%2 preparation and its effect on Leishmania major promastigotes using MTT assay and trypan blue staining and counting slide hemocytometer reviewed and Artemisia absinthium extract IC50 concentration of 50 mg/ml was determined. In addition, macrophages infected with L. major in terms of xoinvivo slide preparation and staining with giemsa after 24, 48 and 72 hours were examined. The Artemisia absinthium extract was compared with meglumine antimoniate. Data on the occurrence, T-test and (Repet Measuer) RQC curve assessment was calculated using SPSS. The results showed that Artemisia absinthium extract on the growth of Leishmania major promastigotes in concentrations 800 and 25.6 mg/ml after 72 hours of anti-Leishmania respectively 97.3% and 9.28% had the killing effect. The effect of anti-Leishmania glucantime was 100% after 72 hours with the concentration 0.003 mg/ml and a minimal effect on the concentration of anti-Leishmania with 0.003 mg/ml after 72 hours was 60%. The effects on amastigotes, Artemisia absinthium extract at a concentration of 800 mg/ml after 72 hours, most of the anti-amastigote (88.8%) have had. at a concentration of 6.25mg/ml after 72 hours (22.2%) and anti-parasitic effect glucantime at concentrations of 0.03 and 0/003 mg/ml after 72 h, 100% and 55.5% respectively.Compared to glucantime, Artemisia absinthium extract had the desired effect in inhibiting the growth of Leishmania major. Therefore, it is suggested to use the plant as a medicine against cutaneous leishmaniasis, and various studies on other species and strains are recommended.

**Keywords:** *Leishmania major*, artemisia absinthium, MTT, Cell culture, Balb/c mouse.

## MOLECULAR CHARACTERIZATION OF RPOIILS ENCODING RNA POLYMERASE II LARGEST SUBUNIT FROM LEISHMANIA MAJOR (MRHO/IR/75/ER)

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Leishmaniasis is a geographically widespread severe disease, which includes visceral leishmaniasis (VL), and cutaneous Leishmaniasis (CL). There are 350 million people at risk in over 80 countries. In the old world, cutaneous leishmaniasis was usually caused by Leishmania major, Leishmania tropica, and Leishmania aetiopicacomplex which 90% of cases is occurred in Afghanistan, Algeria, Iran, Iraq, Saudi Arabia, Syria, Brazil, and Peru. Recently, reports showed that some strains of Leishmania major have internal transcribed spacer 1 (ITS-1) with differential size exhibiting homology with the related gene in a divergent genus of kinetoplastida, the Crithidia. This encouraged us to analyze the mentioned gene in 100 isolates obtained from patients with suspected cutaneous leishmaniasis. After obtaining samples from 100 patients, DNA extraction was performed and ITS-1 was analyzed using PCR-RFLP. These samples were sequenced for verifying their homology. Then, RPOIILS gene was analyzed in the samples that their ITS-1 gene exhibiting homology with the related gene in Crithidia. The Findings showed that 10% of the isolates had ITS-1 exhibiting different size with the routine ones. Sequencing of them showed their similarity to the one from Crithidia fasciculata. RPOIILS gene encoding RNA polymerase II largest subunit analysis showed genetic diversity. This study might also help in solving the problems concerning leishmaniasis outbreak currently faced in Iran and some other endemic regions of the world.

Keywords: leishmaniasis, molecular characterization, RFLP-PCR, Iran





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COMPARSION OF LIPIDS OF SERUM IN LEISH-MANIASIS AND BABESIOSIS IN DOMESTIC DOGS

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The aim of this study was to compare the amount of serum lipids in leishmaniasis and babesiasis in domestic dog. In recent years the effect of serum lipids level on the immune system against the blood parasites has been identified. Since most of these parasites are not able to make the required lipids, they receive them from plasma lipids of host. Canine visceral leishmaniasis is a protozoan systemic disease caused by Leishmania genus, created and replicated in macrophage. Canine babesiosis is a tick-borne disease caused by an intra erythrocyte protozoan of the genus Babesia. Three groups of dogs were examined in this study. Each group was consisted of 10 dogs. The blood samples were analyzed using a commercial kit by auto analyzer and HDL, LDL, cholesterol, and triglycerides were measured. Dogs infected with babesiosis showed a significant increase in total cholesterol in comparison with healthy dogs (P <0.05) in sick dogs a sharp increase in triglycerides and a sharp decline in HDL-cholesterol observed. In Leishmania infected dogs, cholesterol, VLDL and LDL increased in comparison with the control group, while a significant reduction in triglycerides and HDL cholesterol in patients with visceral leishmaniasis observed in comparison with the control group (P <0.01).Plasma lipoproteins has a role in transfering lipids between the tissues and organs. In addition, these lipoproteins take part in innate immunity. These results can be attributed to the use of lipids by parasites, liver problems, and interactions between parasite and host cholesterol normal metabolism and over activation of lipoprotein lipase. In conclusion we can say that changes in serum lipids can help in diagnosis of the disease.

Keywords: babesiosis, leishmaniasis, lipid

## IN VITRO ANTI-PARASITIC EFFECTS OF HY-DRO-ALCOHOLIC EXTRACT OF ARTEMISIA DRACUNCLUS ON DIFFERENT FORMS OF LEISHMANIA MAJOR

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Leishmaniasis include a series of parasitic diseases which are present in a wide spectrum of clinical symptoms such as cutaneous leishmaniasis, muco-cutaneous leishmaniasis, and visceral conditions. Plant extracts and compouds obtained from herbals can provide us with a rich source of medicinal and potent factors. In some countries the native plants are used for treating different forms of infectious cases. Recently, there has been a great progress in treating diseases caused by Leishmania major using herbal medicines. In this study, we investigated the in vitro anti-leishmanial effect, i.e. mainly the hydro-alcoholic extract of Artemisia dracunculus in contrast with Glucantime, an anti-Leishmania agent that is derived from Pentavalent Antimony, on standard strains of Leishmania. Standard strain of L. major cultured in RPMI-1640 containing 10% FBS, along with antibiotic agents were incubated in 24±2 °C. Then in the stationery growth phase the effect of different concentrations of Artemisia's extract were compared with glucantime, which is a chemical compound of Pentavalent Antimony on promastigates of L. major. By counting the number of live cells using vital color such as trepan blue and using MTT coloring method, the effect of our herbal extract was studied in comparison with Glucantime. After using MTT assay, the rate of optical density of the produced color was studied and the obtained color, i.e. Formazan made by parasite was measured by ELISA plate reader. The amount of inhibitory concentration (IC50) which is the 50% of the tested extract was calculated and compared with IC50 of the live cells, which were directly counted. For this purpose, the tests were performed duplicate. Findings obtained using direct cell count by vital color trepan- blue and the results obtained by MTT assay in compared with Glucantime had better antiparasitic effect in concentrations of 20-25 mg/ml. The result showed the increased antiparasitic effect of Artemisia extract on promastigates of L. major standard strains. Our results showed clear in vitro antiparasitic effects of the hydro-alcoholic extract of Artemisia on the promastigot of L. major, although, it is reasonable that more experiments are needed for evaluating further aspects of leishmanicidal action of Artemisia extract in the animal models and human volunteers.

Keywords: Artemisia dracunculus, Leishmania major, Glucantime, MTT, in vitro studies





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A COMPARISON BETWEEN INTRALESIONAL INJECTION OF ZINC SULFATE 2% SOLUTION WITH CRYOTHERAPY AND INTRALESIONAL GLUCANTIME WITH CRYOTHERAPY IN THE TREATMENT OF ACUTE OLD WORLD DRY TYPE CUTANEOUS LEISHMANIASIS: A RANDOMIZED DOUBLE-BLINDED CLINICAL TRIAL

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Zinc Sulfate (ZS) has been used for the treatment of acute cutaneous leishmaniasis (CL) recently in both forms of in vivo and in vitro. The aim of the present study was to compare the efficacy of intralesional injection of ZS 2% solution with intralesional glucantime in the treatment of acute CL. In this double-blinded randomized clinical trial (RCT), 80 cases with acute old world dry type of CL were enrolled in the study. The treatment protocol in the first group consisted of intralesional injection of ZS 2% vials once a week for 10 weeks or sooner in case of complete resolution of the lesions. In the second group, intralesional Glucantime once a week for 10 weeks or sooner in case of complete resolution of the lesions were used. In both groups cryotherapy was performed once every other week for 10 weeks. In ZS versus second group, partial and complete clinical response was observed with fewer injections although this difference was not statistically significant. In addition, we found that the trend of treatment in second group was faster but again it was not significant (partial treatment: hazard ratio= 1.4, CI: 95% = 0.7-2.9; complete treatment: hazard ratio = 1.3, CI 95% = 0.6-2.8). The results of this study showed that the intralesional injection of ZS 2% solution was as effective as glucantime on the healing of the acute old world dry type CL.

**Keywords:** zinc sulfate, cutaneous leishmaniasis, intra lesional injection, glucantime EPIDEMIOLOGY AND CLINICAL MANIFESTA-TIONS OF VISCERAL LEISHMANIASIS AND SPE-CIES IDENTITY IN DOMESTIC DOGS USING ELISA, NESTED –PCR AND HISTOPATHOLOGI-CAL TECHNIQUES IN SOUTHERN DISTRICTS OF KERMAN PROVINCE, IRAN IN 2013

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Visceral leishmaniasis is one of the most important neglected tropical diseases with major veterinary and medical impacts. The present study aimed to assess the prevalence of canine visceral leishmaniasis (CVL) and its associated clinical manifestations in southern districts of Kerman province, southeastern Iran. This study was performed as a cross-sectional descriptive study during 2013. A total of 100 randomly selected homesteed dogs were examined by serological, molecular and histopathological methods. A questionnaire was completed for each dog, recording demographic and clinical characteristics. A 5-ml blood sample was taken from each dog for serological examination. Simultaneously, two biopsy samples of spleen tissues were preserved in 70% ethanol and 10% formalin for further molecular identification of the causative agent and histopathological examination respectively. Informed consent of the dogs were obtained from the owners. Data were recorded in a SPSS software and analyzed by chi-square and p<0.05 was considered significant.Overall 100 dogs of 2-12 years old (57 male and 43 female) were evaluated for the presence of clinical symptoms. The highest infection rate belong to male dogs over 10 years old which was significantly higher than other age groups (P<0.05). Overall, 18 dogs were infected as confirmed by combination of methods including ELISA (N=14), Nested-PCR (n=17) and histopathology (n=15). Altogether, 57% of the dogs showed symptoms and 43% were asymptomatic. Of 57 symptomatic dogs 22.8% were infected, while among 43 asymptomatic 11.8% were infected. Nested -PCR revealed L. infantum as the principal causative agent of CVL in the study areas. The present findings revealed that CVL is relatively high in domestic dogs in southern districts of Kerman province, even higher than main foci in northwestern or southern parts of the country. Domestic dogs are the main reservoirs and represent the major potential source of infection for the sand fly vector. Further investigations are essential for planning future control programs.

Keywords: visceral leishmanisis, Leishmania infantum, dogs, epidemiology, Kerman





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### A SURVEY OF RESERVOIR HOSTS IN TWO FOCI OF CUTANEOUS LEISHMANIASIS IN KERMAN PROVINCE, SOUTHEAST OF IRAN

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In the old world, cutaneous leishmaniasis (CL) is zoonoses and natural vertebrate hosts of CL are mammals. This study was carried out on natural infection rates of Leishmania parasites in reservoir hosts in one new focus of zoonotic cutaneous leishmaniasis (ZCL) and in suspected reservoir in an old focus of ACL in Iran. The sampling of rodents using Sherman traps was carried out and PCR technique was used for detection and identification of Leishmania species in Bahreman district, Kerman province, and southeast of Iran. In addition, the smears were taken from suspicious lesions in stray dogs in the city of Kerman, the center of Kerman province. Simultaneously, pieces of lesion  $(1 \times 1 \times 1 \text{ cm})$  were taken for further histopathological examination. Overall, 25 rodents were collected and identified, including Meriones libycus and Rhombomys opimus. Amastigotes were observed in 33% of the R. opimus by microscopic examination and indentified as L. major by PCR technique. Four suspicious dogs out of 391 stray dogs showed no Leishmania species. To the best of our knowledge, this is the first isolation and identification of L. major from R. opimus in Kerman province, where ZCL has been present in recent years. Therefore, R. opimus is considered as the main animal reservoir host in Bahreman ZCL focus. In ACL focus such as the city of Kerman, dogs had no role in CL infection as reservoir host.

Keywords: reservoir host, cutaneous leishmaniasis, Kerman, Iran

### A COMPARATIVE EVALUATION OF THE CUTA-NEOUS LEISHMANIASIS SEVERITY IN OPIUM ADDICTED PATIENTS AND NON-ADDICTED

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Cutaneous leishmaniasis (CL) is a major public health problem in 98 countries. The incidence of CL and opium addiction has significantly been increased in different Iranian provinces recently. The objective of the present study was to explore the possible relationship between the incidence rate and severity of CL with opium addicted and non-opium addicted as compared with control group. This case control and analytical study evaluates 206 participants (consisting of 101 individuals who were infected with CL (14 Addicted, 87 nonaddicted), 105 people not infected (22 addicted, 83 nonaddicted)) in Bam. Five ml blood samples were taken from patients for hematological, biochemical, and immunological tests and also smear preparation for parasitological examination. All statistical analysis were done by SPSS ver. 20. Oneway ANOVA and chi- square was performed for comparing factors (subgroups). P  $\leq 0.05$  was considered statistically significant. The groups were matched by demographic characteristics. Results showed that parasitological factors in CL positive groups showed significant difference between opium addicted and non-addicted. In hematological examination there was a significant different between hemoglobin in all groups (P=0.001). In immunological examination, there was a significant difference between IL-4 (P=0.003), TGF-B (P=0.004) and IFN-8 (P=0.000) in all groups. In contrast, there was not any significant difference between biochemical factors and subgroups. The chance of contracting ACL due to L. tropica among opium-addicted and non-opium addicted individuals was similar, whereas the severity of the disease in opium addicts was significantly higher than that of nonopium addicts. Moreover, health authorities should be aware of the burden and impact of such disease imposed on the wellbeing of humans.

Keywords: cutaneous leishmaniasis, opium addicted, Kerman





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### TOPICAL TERBINAFINE IN THE TREATMENT OF CUTENEOUS LEISHMANIASIS: TRIPLE BLIND RANDOMIZED CLINICAL TRIAL

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Leishmaniasis is a spectrum of disease conditions with considerable health impacts, caused by different species of Leishmania. This disease is currently endemic in 98 countries and territories in the world. There are many treatment modalities for cutaneous leishmaniasis. The use of topical Terbinafine in the treatment of cutaneous leishmaniasis had been considered. Eighty-eight participants more than two years old with proven acute CL by a positive direct smear were randomly allocated to one of the two study arms: One group received meglumine antimoniate (Glucantime) 20 mg/kg/day IM plus a placebo ointment (Mahan Vaseline) for 20 days. The other group received meglumine antimoniate (Glucantime) 20 mg/kg/day IM plus topical terbinafine 250 mg/day, for 30 days and were monitored closely by dermatologist during the course of the study. Crude regression analysis showed that there was no significant difference between placebo and intervention group regarding partial or complete treatment (partial treatment: HRcrude= 1.1, CI 95%= 0.7 - 1.7; complete treatment: HRcrude= 1.1, CI 95%= 0.8-1.7).Treatment rate was more effective in terbinafine received patients groups than the placebo group. However, this rate depended on the type of lesions. As data indicated ulcerated nodules, papules, and plaque in experimental group have been completely improved two times faster than placebo group. Ulcerated nodules, nodules, and plaque were partially improved faster in those used tebinafine than placebo ointment.

Keywords: leishmaniasis, topical terbinafine, glucantime, Kerman, Iran

### LEISHMANIA INFANTUM INFECTION IN DOGS IN HAMEDAN PROVINCE, IRAN

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Dog is the most important reservoir for human visceral leishmaniasis in Iran. The main aim of present study was to determine the sero-prevalence of canine visceral leishmaniasis (CVL) among stray and owned shepherd dogs in Hamedan province. A cross-sectional study was performed in 2013. Randomly 380 blood samples were collected from 170 stray and 210 owned shepherd dogs in different regions of Hamedan province. All of dogs were asymptomatic. The samples were evaluated for presence of Leishmania infantum antibodies using ELISA. Antibodies to L. infantum were detected in 3.95% of samples. The sero-prevalence rate of infection in stray dogs (6.47%) was reported higher than owned shepherd dogs (1.9%) (P=0.023). There was significant differences among infection rate and age groups (P=0.003); unlike different gender (P=0.11). This is the first report of visceral leishmaniasis in dogs in Hamedan province. Further comprehensive studies in determination of infection rate and predominant Leishmania species in humans, sand flies and animals hosts (wild canines and rodents) is recommended in this region.

Keywords: Leishmania infantum, dog, ELISA, Hamedan





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# THE FIRST REPORT OF LEISHMANIA RNA VI-RUS 2-1 IN IRAN USING RT-PCR

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Leishmania RNA Virus (LRV) has a crucial role in disease severity and complications in leishmaniasis. Double strand RNA virus (ds-LRV1) has been detected in Leishmania (Viannia) parasites in new world. So far, there is just one report of *L. major* infected with LRV2-1 in the old world. A series of Iranian Leishmania species were screened for the Leishmania viruses using dsRNA extraction and amplification of viral RNA polymerase gene partial sequence by RT-PCR. LRV was detected in a *L. infantum* extracting from a visceral leishmaniasis patient and a *L. major* extracting from a *Rhombomys* opimus. The two isolates were sequenced and registered in the GenBank as KP054244 and KP054245, respectively. This is the first report of detecting LRV 2-1 in the Mediterranean region and the first report of detecting LRV2-1 in *L. infantum* worldwide.

Keywords: Leishmania RNA virus, RNA dependent, RNA polymerase gene (RDRP), RT-PCR, Leishmania, Iran

### PHYLOGENETIC ANALYSIS OF LEISHMANIA TROPICA POPULATION, BASED ON THE RIBO-SOMAL DNA INTERNAL TRANSCRIBED SPACER SEQUENCE ANALYSIS IN BIRJAND, EAST OF IRAN

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Southern Khorasan Province, including Birjand, has not previously been known as an endemic area for leishmaniasis. But, it seems that during recent five years a stable focal leishmaniasis has been developed in the area. The present study aimed to investigate the phylogenetic relationship between the L. tropica isolates from Birjand and strains of other Iranian endemic regions. Species identification of sixty clinical preparation smears of cutaneous leishmaniasis patients in this region was performed by ITS1 PCR-RFLP. The PCR fragments of 19 L. tropica isolates were subjected to sequencing using the same PCR primers by both directions. The sequences were deposited in the GenBank database and Phylogenetic trees were generated by using representatives of Birjand county sequences as well as 19 Leishmania tropica ITS1 sequences from other regions of Iran retrieved from Gen-Bank database. Analysis of ITS1 sequence of 19 L. tropica isolates showed seven haplotypes that in comparison to GenBank sequences from other Iranian regions, all East Iranian haplotypes were grouped with strains from foci in a presumed geographical band from north west to south east in one clade and defined as group 1. Southeast and east L. tropica as well as those from a focus (Savojbolagh County) near Tehran in central country clustered as monophyletic population structure while two later foci probably originated from southeast L. tropica. Group 2 consisted of some southwest and all northeast sequences and group 3 included those from a focus in central region. All together, central and southwest L. tropica showed paraphyletic populations. This is the first comprehensive study on population structure of L. tropica in Iran that provide a guideline for further molecular based epidemiological studies and with more discriminative markers.

Keywords: phylogenetic analysis, Leishmania tropica, haplotype, Birjand





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### EVALUATION THE EFFECT OF RUMEX ALCO-HOLIC EXTRACT AGAINST CUTANEOUS LEISHMANIASIS CAUSED BY LEISHMANIA MA-JOR IN BALB/C MICE

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Cutaneous leishmaniasis is an endemic disease in many countries, including Iran. Pentavalent antimony compounds, which are used to treat this disease, have adverse side effects. The aims of this study were to evaluate the effectiveness of the alcoholic extract of Rumex on experimental lesion of Leishmania major in Bulb/c mice. In this study, thirty mice were divided into 6 groups A to F, 5 mice in each group, and then inoculated subcutaneously at the base of the tail with 0.1 ml of active Leishmania major promastigotes standard strain (MRHO/IR/75/ER). Cutaneous lesions were appeared approximately after 3 weeks. Three different concentrations of Rumex seeds alcoholic extract (0.3, 0.5 and 0.9) were used as an injection to Group D-F. Three others groups were considered as controls, group A did not receive any treatment, groups B and C received ethanol alone and glucantime, respectively. All injections were performed 2 times a day for 15 days and every week the lesion diameter was measured in all groups. The number of parasites in the lesions stained smears was examined under a microscope. Information were recorded and analyzed by using one -way Anova test and Tukeys test. The mean diameter of lesions decreased in concentration of 9 mg /ml after 5 week and complete healing was observed in this group. Also the parasitic load decreased significantly in comparison with glucantime injected group  $(p \le 0.05)$ . Conclusions: The results suggest that the effective concentration of the herbs mentioned in this article (9 mg/ml) can be used on human cutaneuos leishmaniasis.

Keywords: cutaneous leishmaniasis, Leishmania major, Rumex, Balb/c mice

# PARASITOLOGICAL, SEROLOGICAL AND MO-LECULAR STUDY ON FELINE VISCERAL LEISHMANIASIS IN CATS OF AZARSHAHR EN-DEMIC REGION, EAST AZERBAIJAN PROVINCE IN 2012-2013

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Visceral leishmaniasis is a parasitic infections and zoonotic disease in the countries of Mediterranean basin and Middle East, including Iran. The causative agent of visceral leishmaniasis in Iran is Leishmania infantum and dogs and canines are main reservoirs. Infections of Leishmania infantum in feline have also been proved. The main objective of this investigation was to screen Leishmania infection in feline and possible role in transmission of the disease to human by parasitology, serology and molecular methods in Azarshahr District, East Azerbaijan province in 2012-2013. Totally 65 cats have been trapped alive from villages of Azarshahr County and their serum samples were subjected to DAT for Leishmania antibodies. Giemsa stained impression smears have been prepared for parasitological examination of spleen and liver tissue. Also liver and spleen samples of the cats have been cultured in NNN medium and also used for PCR. Amongst them 15 (23.07%) were seropositive, 13 (20%) had lower titers than positive and 37 (56.92%) feline were seronegative. The smears were prepared from spleen and liver of all animals examined microscopically and did not show any amastigotes. Parts of the spleens and livers of feline were cultured in NNN media, but Leishmania were not isolated from cultures media. Finally from 65 feline spleen tissue DNA extraction for PCR with using specific primers named k-DNA were done but no band linked to Leishmania was observed.According to serological findings in this study and the endemic diseases in the region, chance of contamination probably will increase if extensisive studies on more obvious clinical signs and also on the vectors of leishmaniasis are carried out.

Keywords: visceral leishmaniasis, cat, DAT, PCR, Azarshahr, Iran





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## EVALUATION OF LEISHMANIACIDAL ACTIVITY OF PHOENIX DACTYLIFERA L EXTRACT AGAINST LEISHMANIA MAJOR (MRHO/IR/75/ER)

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Plant extracts and plant-derived compounds are unlimited sources of chemical diversity for identification of new medicinal agents commonly used to treat infectious diseases. The purpose of this study was to evaluate the in vitro anti leishmanial activity of Phoenix dactylifera L. (Seed and fruit) extracts against L. major promastigotes using colorimetric MTT assay as compared to the pentavalent antimonial compound. The methanolic extracts prepared by percolation method. The extracts were dried and solved in DMSO 5% solvent. L. major promastigotes were cultured at 25±2°C in stationary phase in RPMI-1640 medium complemented with 10% FCS and Penicillin-Streptomycin. Then using MTT assay, the cytotoxic activity of plant extracts was evaluated. The optical density was measured using ELISA reader and IC50 values (50%) inhibitory concentrations) were evaluated. All tests were repeated in triplicate. Plant extracts and glucantime interdicted the growth of promastigote forms of L. major in vitro after 24, 48, 72 hours of incubation. IC50 values for methanolic extracts seed and fruit Phoenix dactylifera L. 23µg/ml, and 500 mg/ml was measured respectively. Seed extract was more effective than fruit Phoenix dactylifera L.extracts, on promastigotes of L. major. As regards, Phoenix dactylifera L. Seed extract showed in vitro anti-leishmanial property, further works are required to appraise the exact effect of these extracts on Leishmania agents in animal models.

Keywords: Leishmania major, Phoenix dactylifera L, MTT

EPIDEMIOLOGICAL FEATURES OF CUTANEOUS LEISHMANIASIS IN MAZANDARAN PROVINCE

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Leishmaniasis is a parasitic disease and unfortunately the number of positive cases of the disease increases each year. In addition, no effective vaccine has been made against the disease. Due to the temperate climate of Mazandaran and abundance of mosquito vectors (sand fly) in this province, the disease has epidemic potential. This study aimed to investigate the process of the epidemiology of leishmaniasis in Mazandaran province.In this descriptive study the data and records kept in the disease prevention department of the office of vice chancellor for health were analyzed in terms of city and type of parasites.During 7-year period from 2007-2013 the total number of cases infected with the disease was 248. The percentage of rural leishmaniasis was 32.5 and 67.5 percent of the cases was found in cities. Behshahr had the highest number of leishmaniasis and Chalus included the lowest number. Most cases of infection were found in the 20-29 (25%) years age group. All the participants received free treatment of Glucantime vaccine which was prescribed by a physician specialized in infectious diseases. Due to the increasing number of leishmaniasis cases in the country and presence of favorable conditions for the establishment of the parasite life cycle in this province, in addition to the presence of patients infected with leishmaniasis as the urban source of the disease and rodents as the rural reservoir, there is the possibility of the onset of an outbreak in Mazandaran. Therefore, through various measures such as broadcasting educational programs through the mass media, the necessary education regarding methods of prevention, transmission and treatment of leishmaniasis should be provide to the public.

Keywords: leishmaniasis, epidemiology, Mazandaran





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WHAT IS THE DIAGNOSIS? CUTANEOUS LEISHMANIASIS OR FISH TANK GRANULOMA?

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Cutaneous leishmaniasis (CL) is still a great health problem in Iran. Clinical presentation of CL is very similar to skin lesions of fish tank granuloma. Differential diagnosis of CL with Mycobacterium marinum infection is important to make decision for proper treatment. In this survey 24 cases with clinically suspected skin to CL, diagnosed as fish tank granuloma (FG) were studied. During 4 years (2011 - 2014), 24 patients admitted to Parasitology Laboratory in special clinics of Mashhad University of Medical Sciences. All of the patients primarily, referred to dermatologist and clinically diagnosed as CL, then referred to the lab to confirm the diagnosis. The skin lesions were erythematic nodules mostly with crust and principally located on the hands and fingers. Pervious history of contact with fish, aquarium water and swimming inside fresh water pools was considered. Direct stained smear with Geimsa and Ziehl-Neelsen in addition to culture on Löwenstein-Jensen medium performed for each patient. Result: All of the patients had skin lesions, clinically very suspected to CL, but direct Geimsa stained smears from their lesions was negative for Leishmania while Ziehl-Neelsen smear and/or culture on Löwenstein-Jensen medium were positive for bacilli of Mycobacterium marinum. More than 90% of the patients were young males with history of manipulating fish inside the home aquarium.Erythematic nodular skin lesions similar to CL lesions specially located on hands with previous history of contact with aquarium water, manipulating fish or swimming inside open pools must be examined for Mycobacterium marinum.

Keywords: Leishmania, Mycobacterium, fish tank granuloma, Mashhad

CANINE VISCERAL LEISHMANIASIS: SERO-PREVALENCE AND PARASITOLOGY SURVEY OF ASYMPTOMATIC DOGS IN MESHKIN-SHAHR DISTRICT, AN ENDEMIC AREA OF NORTH-WESTERN IRAN

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Canine visceral leishmaniasis is a major public health problem that is endemic in tropical and subtropical countries and is fatal in humans and dogs. In addition to symptomatic dogs, asymptomatic ones seem as source of Leishmania infantum infection. Thus surveillance and control programs of reservoir hosts are essential. This study aimed to evaluate the seroprevalence of visceral leishmaniasis in asymptomatic domestic dogs from an endemic area of North West, Iran. A crosssectional study was carried out in Meshkin-Shahr district during 2012-2013. For this purpose, anti-Leishmania antibodies were detected with direct agglutination test (DAT) in 110 asymptomatic dogs. In addition the dogs were examined parasitologically. During the two-year study, of 110 dogs, 22 manifest the disease symptoms (20%) and 88 dogs (80%) still remained symptomless. 20 out of 22 symptomatic dogs (90%) showed anti-Leishmania antibodies by DAT (higher or equal to 1/320.) and of 88 asymtomatic dogs, 16 (14.5 %) dogs showed symptoms gradually, while the rest maintained asymptomatic. Parasitological tests (smears of liver, spleen, skin lesions and culture) in dogs with no symptoms showed that most of the dogs (60%) were positive. The demographics, age, age of incidence and gender were investigated in this study. Most of asymptomatic dogs had no significant titers of antibodies but were positive in parasitological tests.In addition to symptomatic dogs, asymptomatic ones seem to be as source of Leishmania infantum infection, especially dogs with low antibody titre. It is recommended that asymtomatic dogs to be follow up for antibody and symptoms of the disease.

This study was supported by research station of Meshkinshahr of School of Public Health, Tehran University of Medical Sciences.

Keywords: visceral leishmniasis, asymptomatic dogs, DAT, parasitplpgy, endemic





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### ALTERATION OF DIRECT AGGLUTINATION TEST (DAT) RESULTS IN IRANIAN KALA-AZAR PATIENTS: A CASE SERIES

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Early diagnosis using direct agglutination test (DAT), treatment and management of human VL cases are essential actions based on national protocol of Iranian Ministry of Health, which have been employed in the rural health centers at endemic foci. Eleven clinically confirmed VL patients, who admitted to kala-azar laboratory in Meshkinshahr Valiasr Hospital, resulted in negative by direct agglutination test (DAT). So the possible factors involved in false negative test result were notified. The blood samplings were done by finger prick of VL patients admitted to the kala-azar laboratory and blood samples were taken in micro- hematocrit tubes, centrifuged and their plasma separated. The complete history of VL patients was gathered using a written questionnaire. All the patients were referred by specialist's supervision and clinically diagnosed visceral leishmaniasis with typical symptoms but unexpectedly their laboratory DAT test resulted in negative reaction. So, this led to motivate the laboratory staff to check the quality of anti-Leishmania antigen, materials, equipments as well as patients' history. All the patients have been administered Glucantim® as well as corticosteroids drugs (dexametazone /hydrocortisone) before referring to the laboratory. After 2-3 weeks of last injection of the corticosteroids, DAT repeated and the titers surprisingly showed positive trend. The second tests revealed that 3 (27.3%) of the collected specimens were positive with 1:3200 titers, 5 (46%) with 1:1600 and 3 (27.3%) with 1:800 titers.Due to interference of glucocorticostroides with the result of serological diagnosis test, it should be notified the history of drug administration especially glucocorticostroides among VL patients.

Keywords: visceral leishmaniasis, direct agglutination test, false negative, corticosteroid

# ASSOCIATION BETWEEN CLIMATIC INDICATOR AND INCIDENCE OF ZOONOTIC CUTANEOUS LEISHMANIASIS IN KERMAN PROVINCE OF IRAN

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Leishmaniases are among the most important emerging and resurging vector-borne diseases, second only to malaria in terms of the number of affected people. Leishmaniases are endemic in 98 countries and three territories worldwide and threaten about 350 million people. It is estimated that 14 million people are infected worldwide with about two million new cases occurring each year. The parasite is then transmitted to human hosts by the bite of a Phlebotomine sandfly that was previously fed on an infected reservoir. In this paper we aimed to examine the monthly dynamics of the incidence of this zoonotic cutaneous leishmaniasis (ZCL) as a function of surface climatic and environmental indicators in region covering a large area of the semi-arid of southeastern portion of Iran, Kerman province.For achieving this aim, we used monthly ZCL incidence data during 2007 to 2011(5 years) and the monthly mean air temperature data, and 2 product of Modis namely Land Surface Temperature (LST) and Enhanced Vegetation Index (EVI), to monitor land temperature and vegetation cover. Finally, we used correlation and crosscorrelation to investigating the relationship between climate factors and ZCL incidence in Kerman Province. We found strong negative correlation between ZCL incidence air climatic factors simultaneously. But with 5 to 3 month lag in the same year, we observed the strong positive correlation between this variable in Kerman province. We found evidences that changes in climate contribute significantly to the increase in the number of cases and expansion of the range of ZCL as with other vector-borne diseases. Seasonal patterns in the number of cases and vector abundance suggest that ZCL transmission is sensitive to the physical environment.

Keywords: cutaneous leishmaniasis, climate indicators, correlation, Kerman





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## IN VITRO EFFECT OF NICOTINAMIDE ALONE OR IN COMBINATION WITH GLUCANTIME ON PROMASTIGOTE AND AMASTIGOTE STAGES OF LEISHMANIA TROPICA

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Leishmaniasis is a complex disease with a broad spectrum of clinical features, usually divided into cutaneous leishmaniasis (CL), muco-cutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL). Nicotinamide (NAm) has been used for a wide range of applications including anticancer and antidiabetic. This study aimed to evaluate the anti leishmanial effects of NAm against promastigote and amastigote stages of Standard tropica. strain of L. tropica Leishmania (MHOM/IR/2002/Mash2) cultured in NNN medium and subcultured in RPMI 1640 medium, supplemented with 10% fetal calf serum (FCS) and antibiotics then incubated at 25  $\pm$ 2°C. The anti leishmanial effects of NAm alone or in combination with glucantime (meglumine antimoniate, ma) on L. tropica promastigote and amastigote stages were evaluated, using a MTT assay and a macrophage model, respectively. All the tests in this study were carried out in triplicate. Data analysis was carried out by using SPSS version 17.0. Differences between test and control groups were analyzed by ANOVA and t-test. The OD and IC50 values showed that NAm in combination with Glucantime had better anti leishmanial effect than the control drug. The IC50 for the Glucantime was 95 µg/ml, whereas it was 48 µg/ml for NAm in combination with Glucantime and 109 µg/ml for NAm alone. NAm in combination with Glucantime caused significant decrease in the mean infection rate (MIR) and mean number of amastigotes in each macrophage when compared with NAm and Glucantime alone. (P<0.05).As NAm showed a significant anti leishmanial effects on L. tropica promastigote and amastigote stages, further studies are required to evaluate the anti leishmanial effects on L. tropica using volunteer human subjects.

Keywords: Leishmania tropica, nicotinamide, promastigote, amastigote, in vitro.

# A COMPARITIVE STUDY OF THE EFFECT OF VITAMIN K1, MEGLUSAN AND GLUCANTIME AGAINST CUTANEOUS LEISHMANIASIS LE-SIONS IN BALB/C MICE

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Cutaneous leishmaniasis is a variety of protozoan diseases endemic in Iran. As to the adverse effects, long-term treatment, and drug resistance of antiparasitic drugs, the purpose of this study was to examine the possible useful effect of vitamin K1 in the complementary treatment of leishmaniasis. In this case-control study, amastigotes of major Leishmania parasite, strain (MRHO/IR/75/ ER) were inoculated in the foot of 24 Balb/c mice. After 25 days, the lesion appeared in Mice's feet. The mice were divided into 3 groups and treated with standard doses of phylloquinone amp (vitamin K1), Meglusan amp and Glucantime amp for 28 days, interperitonealy. The control group received no treatment. Ulcer lesion size was measured in both groups. Based on paired t-test, the mean effect of Glucantime and Meglusan reduced over time from zero week to week 5. Phylloquinone decreased from zero week to week 3, and then took an upward turn so much so that in the fifth week has reached the baseline level as in the zero week. Average effect of Glucantime was (0.61±-6.33), Meglusan (0.53 + -7.15) and phylloquinone (0.39 + -7.15)6.59). Mean difference of drugs was statistically significant (p < .001). The mean of each of these drugs was not statistically significant with that of the control group. According to the results of this study, it seems that vitamin K1, producing fibrinopeptid and trombin, can be used as a substitute treatment in controlling the lesion in the first three weeks of cutaneous leishmaniasis in the absence of other drugs or the prohibited use of glucantime. Therefore, it is recommended that integrative and separate studies to be done with vitamin K1, Meglusan, and glucantime.

Keywords: Vitamin K1, Balb/c, meglusan, glucantime, cutaneous leishmaniasis





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DESIGNING AND CLONING OF MOLECULAR CONSTRUCTS TO KNOCK OUT GPI12 GENE IN LEISHMANIA MAJOR AS AN EXPERIMENTAL MODEL FOR A CANDIDATE VACCINE

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Leishmaniasis represents a major public health problem in the tropical and sub-tropical countries. At present, there is no efficacious vaccine or effective drugs against the disease .New innovative control method is highly required. One way to achieve this important goal is evaluation of vital and important enzymes, proteins and macromolecules by reverse genetic engineering method .In these types of researches, the gene of specific macromolecules would be deleted for evaluation of the effect of deletion on growth, multiplication and pathogenesis of the parasite. The aim of this study was to design and clone molecular constructs to knock out GPI12 gene in L. major to be used as an experimental candidate vaccine in in vitro and in vivo model. For designing and making molecular construct, we used pLEXSY-neo2 and pLEXSY-hyg2 vectors provided by Jena Bioscience Company (Germany). This vector contains a marker gene that after entering the genome, encodes aminoglycoside phosphotransferase and hygromycin phosphotransferase. Selection of such transfected parasites with constructs containing these markers can be isolated and selected by the Neomycin and Hygromycin antibiotics. Hence, utr2 and utr3 regions along with gene markers were used. Therefore, flank sequences of GPI12 gene, which we intend to knock out, were arranged after these two areas. The molecular construct were cloned in E.coli strain Top10 and confirmed by molecular methods and were transfected by electroporation into L.major. After colony PCR for confirmation of the molecular construct, they were sequenced. The constructs after linear up by enzyme Swa I were transfected by electroporation into the parasite in two stages; the first stage by Neomycin resistance gene cassette containing construct, and secondly by the resistance gene cassette containing Hygromycin constructs and then selecting the recombinant strains by selective antibiotics after which they were confirmed by PCR and Southern blot. Consequently, recombinant parasites were created and examined for subsequent study. With the use of molecular construct the ability to remove and study GPI12 gene was possible to achieve a recombinant Leishmania parasite. This achievement can be used as an experimental model for a vaccine candidate. Further investigations are necessary to check this model in a suitable host.

Keywords: molecular construct, Gene knock out, southern blot, cloning, *Leishmania major*, electroporation

EPIDEMIOLOGIC STUDY OF CUTANEOUS LEISHMANIASIS IN NAZAR ABAD CITY DURING 2010-2014

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Leishmaniasis is a parasitic disease that is caused by Leishmania species. Leishmania infection is endemic in scattered foci in more than 98 countries on five continents, mostly developing countries. Leishmaniasis is transmitted by the bite of sand flies and shows three forms of cutaneous leishmaniasis (CL), visceral leishmaniasis (kala-azar) and muco cutaneous leishmaniasis (MCL). Globally, the annual incidence of cutaneous leishmaniasis is estimated to be 0.7 to 1.2 million new cases per year. In this study, the frequency and epidemiological characteristics of patients with cutaneous leishmaniasis in Nazar Abad were studied. In this retrospective study, a total of 9 CL cases were recorded in the health centre of the city of Nazar Abad during 2010 to 2014. The statistical indices were calculated using SPSS software. From 9 patients 4 were male and 5 were female. Highest cases of infection were observed in age group 47-63 years and the lowest in 20-30 years age group. Sixty percent of the cases were from urban areas and 40% from ruralregions. The majority of the cases presented with sores on the feet, follow by the sores on hands and face.Results showed statistically significant differences between males and females. These results can be used in control and prevention program in future by authorities and executive planners. Promoting knowldge of the disease and methods of health education on personal protection is necessary.

Keywords: cutaneous leishmaniasis, patients, sandflies, epidemiological





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EPIDEMIOLOGIC STUDY OF CUTANEOUS LEISHMANIASIS IN HASHTGERD CITY DURING 2010-2014

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Cutaneous leishmaniasis is one of the most important diseases transmitted by sandfly and has been one of the most important health problems in Iran. Determining the epidemiological aspects of disease is important for planning of control program. This study was carried out to describe the cutaneous leishmaniasis situation in Hashtgerd district. In this retrospective study, 26 recorded cutaneous leishmaniasis cases diagnosed during 2010-2014 in Hashtgerd district, along with demographic data such as age, sex, and sites of ulcer(s), were registered and the statistical indices were calculated using SPSS software. Of 26 under care patients, 10 cases (38.4%) were male and 16 (61.5%) female. Highest cases were found in in age group 20-40(34.6%) years and 40-60(15.38%) years age group had the lowest risk. 23 patients (88.5%) resided in rural areas, while 3 (11.5%) lived in urban areas. Hands and feet were the most common sites of ulcer (85%).Cutaneous leishmaniasis is an endemic disease in desert rural areas and is developing in suburban parts of Hashtgerd; therefore, comprehensive planning for control and prevention of the disease is necessary.

Keywords: cutaneous leishmaniasis, patients, sandfly, epidemiology IDENTIFICATION OF LEISHMANIA ISOLATES BY NESTED POLYMERASE CHAIN REACTION METHOD (NESTED- PCR) IN VARZANEH CITY, ISFAHAN PROVINCE, IRAN

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Leishmaniasis is a parasitic disease caused by the protozoan parasites of the Genus Leishmania, transmitted by sandflies. Cutaneous leishmaniasis (CL) in old world is usually caused by L. major, L. tropica, and L. aethiopica complex. One of the most important hyper endemic areas of CL in Iran is Isfahan province. Varzaneh is a city in the eastern part of Isfahan province. Due to different biological patterns of parasite strains distributed in the region, this study was designed to identify Leishmania species from human victims using Kinetoplastid DNA as templates in a molecular PCR method. Among 186 suspected cases, 50 cases were confirmed positive by direct microscopy after Giemsa staining. Species characterization of the isolates was done using Nested- PCR as a very effective and sensitive tool to reproduce mini circle strands. After Nested-PCR from all 50 cases, 560 bp bands were produced which according to products of reference strains indicated that the infection etiologic agent was L. major. 22 (44%) of patients were females and 28 (56%) males. Their age ranges were between 7 months and 60 years. According to the results of the study and the particular pattern of infection prevalent in the region, genetic studies and identification of Leishmania parasites are very important in the disease control and improvement of regional strategy of therapy protocols.

Keywords: Leishmania, nested-PCR, Varzaneh, Isfahan





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LEISHMANIA INFANTUM FML (FOCUSE MAN-NOSE LIGAND) PULSED-DENDRITIC CELLS IN-DUCE A PROTECTIVE IMMUNE RESPONSE IN MURINE VISCERAL LEISHMANIASIS

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Protection against visceral leishmaniasis (VL) is one of the best control strategies for this vector-transmitted protozoan disease. Dendritic cell (DC) based vaccination is a beneficial tool for inducing antigen-specific immune response. In this study the efficacy of focuse mannose ligand (FML)-loaded dendritic cells (DCs) in protection against VL was investigated. Mice were immunized with FML- or SLA-loaded DCs as well as FML or SLA in saponin and challenged with L. infantum amastigotes. The levels of cytokines before and after challenge were detected by ELISA. Parasite burden (total LDU) was determined after parasite challenge. FML-saponin induced the highest IFN-y/IL-4 ratio among vaccinated groups before parasite challenge, though this ratio was higher in FML-loaded DCs group subsequent to challenge with L. infantum. Moreover, the greatest reduction in parasite number was detected in mice vaccinated with FML-loaded DCs compared to PBS-treated mice (p=0.002).FML-loaded DCs are one of the promising tools for protection against murine VL.

Keywords: dendritic cells, FML, Soluble Leishmania antigen, Leishmania, vaccination, IFN-y, IL-4.

STUDY ON LEISHMANIA INFECTION IN CATS FROM AHAR, EAST AZERBAIJAN PROVINCE, NORTHWESTERN IRAN BY PARASITOLOGICAL, SEROLOGICAL AND MOLECULAR METHODS

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The aim of this study was to study Leishmania infection in cats and its potential role in transmission of the disease to human by parasitological, serological and molecular methods in Ahar District, East Azerbaijan Province. In this study, 65 cats from different parts of Ahar province were trapped. The cats were anesthetized with chloroform and blood samples were taken from jugular vein and tested by direct agglutination test. Spleen and liver smear samples were prepared in order to microscopically examine these organs, and also cultured in Novy-MacNeal-Nicolle and Roswell Park Memorial Institute 1640 media. Finally, spleen tissue DNA was extracted to perform polymerase chain reaction analysis. In direct agglutination test, 4 (6%) cats had a positive titer, while 14 (22%) cats had a titer of 1:80 which was suspected for an infection and 47 (72%) cats were negative. Culture results were negative and in polymerase chain reaction no amplification was observed.We found no case of feline visceral leishmaniasis. It needs more extensive studies by using a larger number of cats to establish the stable diagnosis of leishmaniasis in this area.

Keywords: cat, Ahar, Leishmania





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#### EVALUATING OF FELINE VISCERAL LEISH-MANIASIS IN TWO ENDEMIC FOCI OF IRAN BY FML ELISA

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Leishmania infantum, the causal agent of visceral leishmaniasis in Mediterranean basin, is endemic in south (Fars) and northwest (Ardabil and East Azarbaijan) of Iran. Canids both domestic and wild are regarded as major reservoir hosts for L. infantum. Recently several reports of feline leishmaniasis caused by this organism were reported around the world. Regarding to the high sensivity and specifity of FML-ELISA in serodiagnosis of VL in dogs and human, we prompted to use FML-ELISA for investigation of VL in domestic cats in endemic areas of Iran. Blood samples of 48 cats were collected from two endemic areas of Iran. Fucose mannose ligand and soluble Leishmania antigens extracted from native L. infantum and coated in 96-well-plates. ELISA was performed on cat's sera using FML and SLA antigens. Anti-Leishmania antibodies were detected in 16% (8/48) and 18.75% (9/48) of serum samples by FML and SLA ELISA respectively.Occurrence of L. infantum infection among cats in endemic areas of Iran indicates the potential risk of cats for public health.

Keywords: FML-ELISA, visceral leishmanisis, cat

### CO-INFECTION OF VISCERAL LEISHMANIASIS IN HIV-POSITIVE PATIENTS IN MASHHAD, NORTHWEASTERN IRAN

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Visceral leishmaniasis (VL) infection is a common form of leishmaniasis in human immunodeficiency virus infected patients. VL serosurvey was performed on 49 HIV/AIDS positive patients in Khorasan Razavi province. HIV infections were diagnosed by ELI-SA and confirmatory Western blot assay at the AIDS center of the Khorasan Razavi province. All sera were screened using the direct agglutination test (DAT). The sera with anti-Leishmania infantum antibodies at a titre of 1:100 were considered positive for VL infection and serum titration was done from 1:100 to 1:102,400. Result: Nine (18.4%) patients were sero-positive according to DAT. The distribution of sera titrations were as follows: 1:100 (n= 6) 1:1600 (n= 1); 1:25,600 (n= 1) and 1:102,400 (n= 1). All sero-positive cases demonstrated clinical signs and symptoms. The most predominant signs and symptoms of co-infection of visceral leishmaniasis in HIV-positive patients were pneumonia (n=2), hepatosplenomegaly (n=2), lymphadenopathy (n=2), anemia (n=1), prolonged fever (n= 1) and cachexia (n= 1). Amastigote form of Leishmania sp. was found in bone marrow aspiration materials of one DAT positive patients (titre 1:25,600). Based on nested-PCR assay, L. major was identified in the patient.Our result showed that VL (kala-azar) is an opportunistic disease in HIV-positive patients that may happen in VL endemic areas of Iran.

Keywords: visceral leishmaniasis, HIV, DAT





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# COMPARISON BETWEEN IMMUNE RESPONSES TO SLA INCORPORATED IN CATIONIC IMMUNE STIMULATING COMPLEXES (PLUSCOMS) IN BALB/C MICE

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PLUSCOMs are particulate antigen delivery systems consisting of cationic ISCOM derivatives, and able to elicit in vivo T cell responses against a model protein antigen SLA (Soluble Leishmania antigens). The aim of this investigation was preparation, characterization and assessment of type of immune response generated against leishmaniasis in PLUSCOMs containing soluble Leishmania antigens as vaccine in murine model. Immunostimulatory complexes (ISCOMs) are particulate antigen delivery systems composed of antigen, cholesterol, phospholipid and saponin, but ISCOMs did not incorporate into hydrophilic protein composition, while with cationic charge PLUSCOMs, hydrophilic proteins readily adsorbed onto PLUSCOMs. To induce a Th1 type of response and protection in Balb/c mice against Leishmania major infection, 2-dioleoyl-3-trimethylammonium-propane (DOTAP) 1 PLUSCOMs bearing an intrinsic adjuvanticity, were used as an antigen delivery system and immunoadjuvant for soluble Leishmania antigens (SLA). Balb/c mice were immunized subcutaneously. As criteria for protection, footpads swelling at the site of challenge and foot parasite loads were assessed. The immune responses were also evaluated by determination of IgG subtypes and the level of IFNy and IL-4 in cultured splenocytes. Result: There was no significant difference (P <0.05) between the sizes of the lesion in groups of mice immunized with different formulations. The results of footpad parasite burden confirmed that the level of parasite in group of mice which was immunized with PLUSCOM was significantly (p < 0.05) smaller than all of the other groups. Higher IgG2a/IgG1 ratio was seen in this group which is an indicator of Th1 response. Antibody assay showed that PLUSCOM/SLA induced the highest IgG2a/IgG1 ratio at all dilutions. Also, IgG2a/IgG1 ratio after challenge showed that PLUSCOM+SLA induced the highest IgG2a/IgG1 ratio in all dilutions. The results of cytokine assay demonstrated that the highest level of IFN-  $\gamma$  secretion was observed in the splenocytes of mice immunized with PLUSCOM/SLA and lower amounts of IL-4 was observed in PLUSCOM group as compared to buffer. Results indicated that soluble Leishmania antigens in different formulations generated an immune response with mixed Th1/Th2 response that was not protective despite the activation of large numbers of CD4+ T cells with secreting IFN-γ.

Keywords: PLUSCOMs, ISCOMs, Quil A, Leishmania major, immune response

HIGH INFECTION FREQUENCY, LOW DIVERSI-TY OF LEISHMANIA MAJOR AND FIRST DETEC-TION OF LEISHMANIA TURANICA IN HUMAN IN NORTHERN IRAN

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Identifications of Leishmania species are important because of clinical and epidemiological reasons. As a result of morphological similarities, Leishmania species could not be firmly identified using conventional methods. The first objective of our research is to perceive the possibility of L. major detection in humans and also characterization of Leishmania parasites in a well known endemic ZCL of Iran. The last, is to find out the duration feasibility of mammals Leishmania species in human such as L. turanica, L. gerbilli and Leishmania species near L. gerbilli which were found previously in Iranian Phlebotomus and/or in rodents. There is no record for detecting L. turanica and L. gerbilli in human. Smears of suspected patients infected with zoonotic cutaneous leishmaniasis (ZCL) were stained and examined under a light microscopic. DNA of parasites within human ulcers was extracted directly from their smears. Nested PCR was used to amplify a fragment containing the internal transcribed spacers of the ribosomal RNA genes (ITS-rDNA). The ITS1-5.8S rRNA fragment was 480 bp (including primers) which were subjected to nested PCR, RFLP and Sequencing. MEGA 5.05 software was used for phylogenetic analyses. 128 suspected patients of 164 individuals were Leishmania positive by amplifying ITS-rDNA fragment in agarose gel 1.5%. 125 Leishmania positive out of 128 were digested by BsuRI (HaeIII) and sequenced to identifying species and characterization of Leishmania parasites. Three out of 128 samples did not have enough DNA to digest by BsuRI (HaeIII) and/or sequence. 123 of 125 positive samples were firmly established to identify as L. major and two others as L. turanica for the first time.Two suspected isolates were again examined by Nested PCRPFLP and sequenced several times. The sequences finally was again identified as L. turanica, haplotype TurkH03 (GenBank accession no. EF413078) which previously isolated and identified from sandflies and rodents in this region and elsewhere.In our current investigation of Leishmania detection in humans of Turkemen Sahara in Golestan province, L. major (98.4%) and for the first time L. turanica (1.6%) were identified. L. major was high in density with 123 out of 125 and L. turanica had low density with two amplified samples. It was believed that L. turanica only survive in their rodents' host reservoirs and sandflies until now but our research revealed that two cases of L. turanica can be associated with various types of presenting lesions among intraspecies Leishmania parasites from infected patients.

Keywords: Leishmania major, Leishmania turanica, human, ITS-rDNA, Iran





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### IN VITRO EFFECT OF A HERACLEUM PERSI-CUM AND PUNICA GRANATUM MIXTURE ON MOTILITY OF LEISHMANIA MAJOR PRO-MASTIGOTES

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Cutaneous leishmaniasis caused by Leishmania major is a common health problem in some parts of the world, including Iran. The pentavalent antimonials, Pentostam and Glucantime are the first line treatment for leishmaniasis. However, resistance to these drugs is a serious problem. This study was conducted to evaluate in vitro effect of different concentrations of a mixture of Heracleum persicum and Punica granatum extracts on promastigote of L. major. In this experimental work, increasing concentrations of the mixture (0.156, 0.312, 0.624, 1.25, 2.5, 5.0 and 10 mg/ml) were added to microtubes containing about 5x105 promastigote of L. maior (MRHO/IR/75/ER), cultured in RPMI-1640 medium. A microtube containing L. major promastigotes and Glucantime was considered as positive control and another microtube only contained L. major promastigotes as negative control. After a 72hr. incubation period, the number of motile promastigotes contained in the microtubes was counted by hemocytometer. After a 72 hours of incubation, no motile promastigote was observed in the microtube contained 10 mg/ml of the mixture. However, in microtubes contained 5, 2.5, 1.25, 0.625, 0.312, and 0.156 mg/ml of the mixture, the anti-motility effect was 83%, 64%, 56%, 44%, 35%, and 19%, respectively. Conclusions: According to the results and some of similarities present in promstigotes and amastigotes of Leishmania spp., it is suggested that in future experiments, the effect of this mixture be investigated on amastigote- infected macrophages, the animal models and finally, human volunteers.

Keywords: Heracleum persicum, Punica granatum, Leishmania major, promastigote

### COMPARATIVE STUDY FOR DIAGNOSIS OF CA-NINE VISCERAL LEISHMAINAISIS USING IFA AND NESTED-PCR IN THE ENDEMIC AREA OF IRAN

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Canine visceral leishmaniasis (CVL) is a severe disease of humans and dogs with more than 90% mortality if not treated. Leishmania infantum is causative agent of CVL in the countries of the Mediterranean basin and Iran. Domestic dogs are the principal reservoir of L. infantum and can be an important threat to public health. In the present study, the sensitivity of a serological method, immunofluorescence assay (IFA) was compared with Nested-PCR to detect CVL in domestic dogs in the endemic area. Sera of 102 household dogs were collected in the present study. The clinical signs of disease, such as peripheral lymphadenomegaly, splenomegaly, papular dermatitis/onychogryphosis, ulcerations, and weight loss were recorded. Five ml of peripheral blood were taken from the foreleg vein of each dog in EDTA for isolating parasite DNA for PCR test, and 2 ml for separation of sera for IFA. The samples were stored at  $-20^{\circ}$ C. Comparison between the results was performed using t-test. Forty one out of 102 (40.2%) dogs were seropositive at >1:320. Twenty one out of 102 (20.6%) dogs were nested-PCR. We found difference between seropositive using IFA with PCR positive. From 29 dogs which were seropositive just 16 (55%) dogs were PCR positive.Our results showed that sensitivity of IFA is higher than nested-PCR in using peripheral blood of domestic dogs in the endemic area.

Keywords: canine visceral leishmaniasis, IFA, Nested-PCR





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PHYLOGENETIC ANALYSIS OF LEISHMANIA TROPICA ISOLATES FROM RURAL AREAS OF HERAT PROVINCE, WESTERN AFGHANISTAN

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Afghanistan is one of the main foci of cutaneous leishmaniasis (CL) in the world. However, no enough studies had been achieved in the endemic areas of CL in this country. Thus, the present study aimed to determine the haplotypes of CL causative agents in rural areas of Herat province, western Afghanistan and to compare their genomic relationship with Iranian isolates. In total, 21 Giemsa-stained slides of rural residents of Herat province referred to WHO centers in a two year period were studied. DNA was extracted from the slides and a partial ITS1 fragment amplified by PCR. The PCR fragments were subjected to sequencing using the same PCR primers by both directions. The sequences were deposited in the GenBank database and analyzed for phylogenetic relationship with other L. tropica isolates based on published data in GenBank. All 21 isolates were Leishmania tropica. Analysis of ITS1 sequence of these isolates showed 4 haplotypes that in comparison with GenBank sequences three of them were in complete agreement with haplotypes recorded in Iran. These isolates were clustered in a clade together with east (Birjand county), and also south-east (Bam and Kerman counties) and Central (Savojbolagh) L. tropica isolates in Iran, and have differences with other isolates from this country. The existence of phylogenetic relationship between L. tropica from Herat and Iranian isolates could help in understanding the epidemiology, transmission and corridor of extend of the disease. Moreover, it is useful in planning of the policy of control and treatment of cutaneous leishmaniasis in these areas.

**Keywords:** *Leishmania tropica*, phylogenetic relationship, haplotype, ITS1, Herat province

# EVALUATION OF 21 KDA ANTIGEN OF LEISH-MANIA INFANTUM IN WESTERN BLOT FOR DI-AGNOSIS IMMUNIZED DOGS FROM UNVAC-CINATED DOGS

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Leishmania infantum (L. infantum), a causative agent of visceral leishmaniasis in humans and dogs, is an obligate intracellular protozoan parasite of mammalian macrophages. An attenuated line of L. infantum (H-line) has been established by promastigotes culturing in vitro under gentamicin pressure as a vaccine candidate. The aim of this study was to confirm the immunogenicity of 21 kDa antigen of promastigotes of L. infantum H-line as a diagnostic marker to distinguishing between the dogs vaccinated with the attenuated parasite with dogs naturally infected with L. infatum wild-type (WT) using Western blotting (WB). Sera from 25 dogs in 3 groups were used in the present study. In group 1, the dogs were vaccinated with the attenuated line and the group 2 dogs were infected with L. infantum WT and in the third group, challenged dogs, and were infected with wild-type parasite with prior vaccination collected. WB analysis was applied using lysates of stationary phase promastigotes of L. infantum H-line and WT. Sera from vaccinated dogs with Leishmania infantum Hline recognized 21 kDa antigen of promastigotes of L. infantum H-line but not of L. infantum WT. Sera from challenged dogs with prior vaccination recognized the 21 kDa antigen of Leishmania infantum WT and L. infantum H-line. Sera from challenged dogs without prior vaccination recognized the 21 kDa antigen of L. infantum WT but not of L. infantum Hline. These results provide a diagnostic technique for distinguishing between dogs vaccinated with L. infantum H-line and dogs challenged with Wild-Type parasite that can be applied for field trial of vaccine against CVL in dog.

Keywords: Leishmania infantum, visceral leishmaniasis, western blot, Iran





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# GENETIC DIVERSITIES OF LEISHMANIA STRAINS ISOLATED FROM CUTANEOUS LEISHMANIASIS PATIENTS IN FARS PROVINCE, SOUTHERN IRAN

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Cutaneous leishmaniasis is present in Fars province in south of Iran. The current study aimed to evaluate the genetic diversities of Leishmania species isolated from leishmaniasis patients in Fars province, using PCR-based analysis and DNA sequencing of N-Acetylglucosamine-1-Phosphate Transferase (nagt) gene. Samples were taken from the skin lesion of 120 suspected CL patients referred to the Shiraz main health centers. A part of each sample was used for in vitro cultivation. DNA was extracted from the cultured parasites and the nagt gene was PCR-amplified. PCR product of nagt gene was digested with Acc1 restriction enzyme for RFLP analysis. Moreover, PCR products of 23 isolates were sequenced and analyzed. From 120 clinically suspected CL patients, 110 (91.7%) cases were found to be positive by direct microscopy while 77 (64.1%) of cultures were positive. Digestion of PCR product with the Acc1 restriction enzyme detected Leishmania major in 57 out of 77 (74.1%) and L. tropica, in 20 out of 77 (25.9%) CL cases. Phylogenetic analysis grouped Leishmania isolates into three main clad, representing L. major, L. infantum and L. tropica, encompassing 2, 2 and 2 haplotypes, respectively. Within the clad, L. tropica intraspecies divergence was more pronounced in L. major. Findings of this study demonstrated that the causative agent of CL in Fars province is mainly L. major and there is considerable heterogeneity between Leishmania species and also within the L. major isolates.

Keywords: Leishmania, genetic variation, Fars, Iran

# EVALUATION OF OSTRICH AND CAMEL SE-RUMS AS ALTERNATIVE TO FETAL BOVINE SE-RUM (FBS) FOR IN VITRO CULTIVATION OF PROMASTIGOTE FORMS OF LEISHMANIA MA-JOR AND LEISHMANIA TROPICA

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RPMI 1640 medium has been applied commonly for culturing of some protozoa such as promastigote of Leishmania. Fetal bovine serum is critical compound of this medium but is very expensive and is not found everywhere. So, in this study we substituted ostrich and camel serums instead of it and growth rate of promastigotes in these media was compared with standard culture medium. RPMI 1640 medium was supplemented with 2.5, 5, 10, 15, 20 and 30 percent of heat-inactivated fetal bovine serum (FBS) or ostrich and or camel serums with 1% of pen/strep antibiotic. About 1x106 promastigotes/ml of Leishmania strain major. MRHO/IR/75/ER and Leishmania tropica, strain MHOM/IR/02/MASH10/ACC were separately cultured in RPMI 1640 medium with different concentrations of these serums in triplicate tubes. Finally, the number of parasites in various days were counted and compared. Our results showed that with increasing of fetal bovine serum concentration until 7 days, the number of promastigotes elevated too. Therefore, RPMI 1640 culture medium containing 30% fetal bovine serum is more effective than those supplemented with 2.5, 5, 10, 15 and 20% of FBS. Parasite reproduction in culture media contained camel serum is completely different as its proliferation reduced with increasing serum concentration. Hence, in low concentration of camel serums, growth speed was better than those enriched under high serum conditions. Propagation rate of promastigotes in ostrich serum media had direct relationship until 10% while in higher serum concentrations, growth reduction observed.RPMI 1640 medium containing low concentration of ostrich and camel serums can be used as effective alternatives up to 96 hour for sufficient growth of promastigotes of Leishmania major and L. tropica.

Keywords: Leishmania major, Leishmania tropica, fetal bovine serum, Ostrich, camel





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### ANTI LEISHMANIAL ACTIVITY OF VIOLACEIN EXTRACTED FROM JANTHINOBACTERIUM LIVIDUM BACTERIA IN VITRO

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The aim of this study was to investigate the anti leishmanial activity of violacein extracted from Janthinobacterium lividum bacteria in vitro. 80 ml of human blood were collected, and their monocytes were extracted with lymphodex, washed 3 times with PBS and cultured in cell culture plates using RPMI 1640 supplemented with 10% FBS for 6 days in 37°C and 5% CO2. Leishmania major parasite (MRHO/IR/75/ER) were cultured in NNN medium for 4 days and after that passaged to RPMI 1640 medium with 10% FBS for 6 days in 25°C incubator. In this phase the parasites were in stationary phase and monocytes changed to macrophages and lost their cycle form. L. major were counted with homocytometer and with 7:1 Ratio parasite: monocyte, the cell were infected and incubated in 37°C with 5% CO2. During 24 hours incubation period, nearly all attached cells got infected and were ready for treatment with violacein. 5 tandem concentration of drug  $(1, 11, 22, 33 \text{ and } 44 \,\mu \text{gr/ml})$  added to infected cells and incubated for another 24 hour. To calculate the ED50, cells were stained with Giemsa and for each concentration live parasites counted in 100 macrophages. The results indicated that violacein have anti leishmanial activity at different concentrations, and the highest concentration of drug (44 µgr/ml) could induce most growth inhibition of parasites in an 80% growth inhibition and the concentration of (33 µgr/ml) of drug indicated as ED50.Different concentrations of the drug had direct effect on growth inhibition of parasite.

Keywords: Leishmania major, violacein, Janthinobacterium lividum

### THE PLANT EXTRACT ACTIVITY STUDY FOR CONOCARPUS, ZIZIPHUS, EUCALYPTUS OLIDA, ALLIUM HIRTIFOLIUM, DESCURAINIA SOPHIA, POMEGRANATE SKIN ON LEISHMANIA MAJOR PROMASTIGOTES, IN VITRO

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The treatment of diseases with plants is as old as history of existence of human being on the Earth. In our country, Iran, the use of herbs has also been common in the treatment of diseases. The plants had also been used by Avicenna known as the "Father of Early Modern Medicine" in the curative treatment of diseases. The purpose of this study was to evaluate the effect of 6 plant extracts on Leishmania major promastigotes in compare with Maglosan (5-valent antimony compound) as standard drug through colorimetric method (Colorimetric assay) of MTT. Leishmania major promastigotes were mass cultured in RPMI 1640 and BHI medium at 27°C. Different dilutions of plant extracts and drug control were added to 96-well plates with designed calculated promastigotes. 24, 48, and 72 hours later, Tetrazolium salt was added to the wells. Attract degree light of Tetrazolium recovery and convertion it to insoluble formazan dye was measured by ELISA Reader. Eventually, the Promastigote viability and the IC50 of extracts and drug control were determined. The experiments were carried out for 72 hours and the results showed that the maximum effect of anti-Leishmania was documented for the Allium stipitatum extracts with an IC50 of 201 micrograms per milliliter, however, the minimum efficacy was recorded for Conocarpus erectus extract with an IC50 of 284 micrograms per milliliter. In addition, the IC50 drug control was calculated at 60 micrograms per milliliter. All extracts showed fairly favorable anti Leishmania properties in vitro and further studies on animal models are recommended.

Keywords: hydroalcholic extracts, Conocarpus erectus, Allium stipitatum, Leishmania major, promastigote, IC50, MTT





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# IDENTIFICATION AND ISOLATION OF LEISH-MANIA INFANTUM 21 KDA ANTIGEN

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Previously, it has been reported that the immunogenicity of 21 kDa antigen of promastigotes of Leishmania infantum (L. infantum) is a diagnostic marker to distinguishing canine visceral leishmaniasis (CVL) in dogs. It was isolated initially from L. infantum stationary phase promastigotes, using affinity chromatography on protein A-Sepharose column. Using Affinity chromatography for separation 21 kDa antigen was on the basis of a reversible interaction between antigen and polyclonal antisera collected from dogs infected with wildtype parasite. Four German shepherd dogs were infected with L. infantum and their sera were collected. The specific anti-Leishmania antibody raised and the levels of antibodies were measured using immunofluorescence assay (IFA). Sera were incubated with lysate of promastigotes at 4°C overnight. Affinity-purified anti-21 kDa antibodies were isolated by low pH elution of antibodies from immunoblots. The sample was then analyzed by SDS-PAGE. This band was confirmed using western blotting. Result: Sera from dogs infected with L. infantum recognized 21 kDa antigen of promastigotes of L. infantum WT. The band has been cut from SDS-Page gel and sent to Proteomics Center for identification and sequencing.

Keywords: Leishmania infantum, chromatography, dog, 21 kDa antigen

### A COMPARISON OF LEISHMANIASIS LABORA-TORIAL DIAGNOSIS METHODS IN LARESTAN DIAGNOSTIC CENTERS

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Leishmaniasis, is one of the most important parasitic-skin diseases in different parts of the world including Iran. This disease impose too much physical injury and financial damage on inhabitants of the region annually. Leishmaniasis is considered as an endemic disease in south of Iran and Larestan, but this disease has reached 549 cases in 2005 from 67 cases in 2003. The increase of sensitive and non-immune subjects is considered as a factor of the outbreak. But the other point is the change of laboratorial diagnosis method of this disease in health centers of the city. The objective of this research was to investigate the effect of this factor. This is a qualitative survey carried out using questionaires and oral survey in all of the diagnosis center (including 10 centers). In this study, the quality of the test and diagnostic knowledge of personnel has been considered. The results showed one of the most important diagnostic centers in the city has changed the method of sampling. They have done sampling from the center of the sores and this issue has resulted in increasing reports of the disease. Taking part of the personnel in retraining courses increased their knowledge of the diagnosis and this is one of the acheivements of this study. Although the increase in number of sensitive persons due to new births is an important factor in outbreak of cutaneous leishmaniasis, other factors also will increase the rate of this disease. Personnel care while watching the cases and the increase in knowledge and efficiency of people and have been probably effective in increase of the diagnosed cases. On the other hand, lack of correct laboratorial diagnosis of the disease can increase the amount of unknown infected persons, so this persons act as a source of disease in the region. Contagious diseases in the region can be controlled by retraining personnel and improvement of the diagnostic methods.

Keywords: leishmaniasis, diagnosis, Larestan.





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IN VITRO ANTI-LEISHMANIAL EFFECTS OF METHANOLIC EXTRACT OF PISTACIA VERA AND PISTACIA ATLANTICA AGAINST LEISHMA-NIA TROPICA

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Leishmaniasis is a complex disease with a broad spectrum of clinical features, usually divided into cutaneous Leishmaniasis (CL), muco-cutaneous Leishmaniasis (MCL), and visceral leishmaniasis (VL). In the present study, we evaluated the anti-leishmanial effects of methanolic extract of Pistacia vera and Pistacia atlantica on in vitro susceptibility of promastigote and amastigote stages of Leishmania tropica in comparison with glucantim (MA, Glucantime) as control drug. Promastigote stages of standard L. tropica (MRHO/IR/75/ER) were transferred to RPMI-1640 medium, supplemented with 10% fetal calf serum (FCS) and antibiotics then grown at 25±2°C. The anti-leishmanial effects of methanolic extract of Pistacia vera and Pistacia atlantica in comparison to MA on L. tropica promastigotes and amastigote stages were evaluated, using a MTT assay and in a macrophage model. The IC50 values (50% inhibitory concentrations), mean infection rate and mean number of amastigotes in each macrophage were determined accordingly. The finding of OD and IC50 showed methanolic extract of Pistacia vera and Pistacia atlantica had better anti-leishmanial effect than the control drug. The IC50 for the glucantim was more than for Pistacia vera and Pistacia atlantica. In addition, methanolic extract of Pistacia vera and Pistacia atlantica caused significant decrease in mean infection rate and mean number of amastigote in each macrophage compared with positive control (P<0.05). As Pistacia vera and Pistacia atlantica showed a significant anti-leishmanial effects on L. tropica promastigote and amastigote stages, further works are required to evaluate the anti-leishmanial effects on Leishmania tropica using volunteer human.

Keywords: Leishmania tropica, Pistacia vera, Pistacia atlantica, promastigote, amastigote

EVALUATION OF PCR AND DAT METHODS FOR THE DIAGNOSIS OF CUTANEOUS LEISHMANIA-SIS USING BLOOD OF PATIENTS IN SHIRAZ, 2014

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Cutaneous leishmaniasis is one of the most important health problems in Iran. Different species of Leishmania parasites cause different clinical pictures and cutaneous form is more prevalent than visceral form. Different methods are now being used for diagnosis. In this study we aimed to evaluate PCR on Buffy coat and DAT (Direct Agglutination Test) for diagnosis of cutaneous leishmaniasis. In this study 30 patients with cutaneous leishmaniasis and 19 healthy subjects as negative controls were studied. 10 ml of blood from each individual was collected, 5 ml of this used for serum preparation and the rest for Buffy coat preparation. The PCR assay was applied on Buffy coat. DAT test was performed using Leishmani major and Leishmania infantum antigens for antibody detection from serum samples. Out of 49 samples with PCR assay, 13 were positive, the rest of patients and healthy subjects were negative. Out of all samples 8 with  $\geq 1$ : 400 antibody titers were positive. In this assay, no cross-reactivity was observed between the serum of patients with toxoplasmosis and malaria. These results suggest that the PCR test has a higher specificity than DAT. Identification of DNA in patients with defect in immune system and with long duration of cutanoues leishmaniasis is more difficult indicating risk of recurrence. DAT result showed that cellular immunity in CL form is more important than humoral and it is not recommended for diagnosis.

Keywords: cutaneous leishmaniasis, PCR, DAT





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THE ROLE OF ACTIVE CASE-DETECTION IN FINDING ANTHROPONOTIC CUTANEOUS LEISHMANIASIS PATIENTS IN AN ENDEMIC FOCUS IN SOUTH EASTERN IRAN AND IDENTI-FICATION OF THE CAUSATIVE SPECIES AND PHYLOGENETIC RELATIONSHIP OF ISOLATES BY PCR-RFLP AND SEQUENCING

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Cutaneous leishmaniasis (CL) is still a public health problem in Iran and other parts of the world with significant increased in the number of human cases. The aim of this stdy was to explore the role of active case-detection in finding anthroponotic CL (ACL) in an endemic focus in south eastern Iran and identification of the causative species and phylogenic relationship of isolate by PCR-RFLP and sequencing. This study was performed as a descriptive- analytical in 2014. Prior to initiating the study, a list of referral patients with CL to the leishmaniasis treatment tlinic was analyzed. We found that most of the CL patients come from 4 areas including Salsabil, Firoozabad, Sarasiab and Sharake- Sanati. Then, a house- to- house visit was planned to systematically assess the total household population. A questionnaire was completed for each individual recording demographic and clinical status. A whole body examination was carried out for presence of suspected active lesion or scar. The suspected individuals were referred to the laboratory, where a smear prepared from their lesions, fixed with methanol and stained by Gimsa for microscopic examination and detection of amastigotes. DNA was extracted from smear for identification of the causative species by PCR- RFLP, using ITS- rDNA. PCR products were sequenced for further bioinformatic analysis of isolates and phylogenic relationship. A total of 3105 individuals with mean age of 26.2 y, comprising 1610 males (51.9%) and 1495 females (48.1%) were examined for the presence of skin lesions. Overall, infection rate was 9.5%, equally divided among genders. Hands were the most site of involvement (49.5%), followed by face (35.3%), legs (9.5%) and the other part of the body (5.7%). About 60% of the cases were found by active case-detection and the remaining (40%) by passive case-finding approach. Majority of patients had single lesion (80.7%), (12.9%) double lesions and (6.4%) had developed three or more lesions. Based on PCR-RFLP analysis, the parasites isolated from the lesions were characterized as L. tropica. Anthroponotic CL due to L. tropica is the only species found in the district of Kerman. Active and passive case-detection strategies should be in high priority to find the total cases by the health surveillance system personnel. Further investigation are required to discriminate the main sand fly vector.

Keywords: cutaneous leishmaniasis, *Leishmania tropica*, epidemiology, PCR-RFLP, phylogeny. DETERMINING THE PREVALENCE OF ALEPPO BOIL (CUTANEOUS LEISHMANIASIS) IN PA-TIENTS REFERRED TO AHVAZ HYGIENIC CEN-TERS DURING 2011-2014

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In 1885, Koningham isolated and identified the Aleppo boil causative agent from skin lesions in India. Aleppo boil or cutaneous leishmaniasis is the most prevalent kind of skin disease that is transferred to human by female sandfly biting. Cutaneous leishmaniasis is one of the greatest hygiene problems in Eastern Mediterranean. Iran is among the six countries where Aleppo boil is one the most prevalent kind of infectious disease. (Iran, Iraq, Saudi Arabia, Sudan, Tunisia, Syria). This study is performed to determine the prevalence of Aleppo boil based on year, season, location, urban or rural people. In this retrospective study, recorded information in registries in center of Aleppo boil diagnosis in eastern hygiene center of Ahvaz were used from 2011 to 2014. All of the recorded information including the number of positive slides divided into gender, season, year, location of mosquito bite, urban and rural people were analyzed considering the privacy of people information. Among 135 positive slides, 90 people (68%) were male and 45 (32%) were female. With respect to disease distribution according to season, average rate in spring was 9.8%, summer 3.3%, fall 31.7%, and winter 54.2%. The prevalence in 2011 was 16.3%, in 2012 43.7%, in 2013, 22.2% and in 2014 was 17.8%. 11.8% lived in rural areas and 81.2% in urban areas. 49.6% of location of bites was in hands, 19% on foot, 9.6% on face and 21.8% was on hands and face or on hand and feet or neck, loin and hand.With respect to the results and using statistical analysis, with 95% probability, we can say that the prevalence of leishmaniasis is in association with gender, it is more reported in male which may be due to their more presence in outdoors or having less personal hygiene. With 95% probability, disease is also associated with season, most cases have been recorded in winter, while the lower numbers seen in summer which means that the heat has advers affect on the disease, in fact parasite growth was high in low temperatures. Presence of more lesions on the hands might be due to the sandfly behaviours.

Keywords: cutaneous leishmaniasis, Aleppo boil, gender, Ahvaz





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### THE STATUS OF RE-INFECTION CUTANEOUS LEISHMANIASIS IN SKIN AND LEISHMANIASIS RESEARCH CENTER IN SEDIGHE TAHERE, IS-FAHAN

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Although, cutaneous leishmaniasis is a self limited disease and not inducing a major problem in terms of death and disability compared to other diseases, however, disfiguring permanent scars, long-term period of wound healing, secondary infection and drug side effects and impose of financial losses have increase the impact and the burden of this disease. This study was designed to investigate the status of cutaneous leishmaniasis reinfection in patients referred to Skin Disease and Leishmaniasis Research Center (SDLRC), Isfahan, Iran. Demographic information (age, sex, number of lesions,) of the patients with cutaneous leishmaniasis referred to SDLRC parasitologic laboratory in the years of 2012 and 2013 was collected. Data was analysed using SPSS version 16. Of the total number of 1087 patients who diagnosed to have cutaneous leishmaniasis, Seventy five patients (6.8%) had reinfection. Mean age of these patients was  $34.73 \pm 20.91$ (ranging 1-75 years). More women (41, 54.7%) were affected than men (34, 45.3%) with no statistically significant difference.Some patients with the history of cutaneous leishmaniasis may suffer reinfection after several years of recovery. Less than 10 % of patients had this problem in the studied population. Several factors such as genotype or strain variations and immunodeficiency due to chronic backgrounds may be involved in the process of reinfection. Further research is warranted to explore the underlying pathophysiology.

Keywords: cutaneous leishmaniasis, re-infection, leishmaniasis

#### SEROLOGICAL DIAGNOSIS OF CUTANEOUS LEISHMANIASIS BY AN IMMUNOBLOTTING SYSTEM

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Diagnosis of cutaneous leishmaniasis (CL) is mainly based on microscopically detection of the parasite, but serological methods might be useful for diagnosis as well. This study aimed to assess the usefulness of an immunoblotting system for diagnosis of CL in Iran.Sixty one serum samples from parasitologically confirmed CL patients and 50 serum samples from healthy controls along with 50 sera from non-CL patients were collected. Native strain of amastigotes of Leishmania major was cultured and soluble Leishmania antigens were prepared from in-vitro cultivated amastigotes-like parasite. All of sera samples were evaluated by a western blotting system. From 61 sera of CL patients, 59 cases (96.7%) detected a 63 kDa subunit and 51 cases (83.6%) recognized a 32-35 kDa component. Among all subunits, the 63 kDa band showed the highest sensitivity (96.7%) and a 75 kDa band had the highest (98%) specificity. Findings of this study revealed that the immunoblotting had a satisfactory performance in diagnosis of CL and this test can be used as an aid for proper diagnosis of CL.

Keywords: cutaneous leishmaniasis, immunoblotting, diagnosis





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ANTI LEISHMANIAL ACTIVITY OF LEAF, FRUIT AND LEAF FRACTIONS OF JUNIPERUS EXCELS ON L. MAJOR BY IN VITRO AND IN VIVO MODEL

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Leishmaniasis is a parasitic disease caused by the parasites of the Genus of Leishmania. The annual incidence of the disease is 1.5-2 million and 350 million people are at risk of the disease. Currently, common choice drugs for leishmaniasis are antimonial and Amphotericin B. The high cost and long term of treatment as well as toxicity, side effects, relapse and resistance to these drugs results in more efforts for new drug discovery. Iran is one of the endemic regions for leishmaniasis, Juniperus excelsa M.Bieb is another choice for anti leishmanial activity. The anti leishmanial activity extracts of leaf, fruit and fractions (petroleum ether, chloroform, ethyl acetate, n- butanol) of leaf of j. excelsa were studied by in vitro and in vivo model. In vitro model, for generation of axenic amastigote, promastigotes were incubated at 33-34°C in Schneider's Drosophila medium supplemented with 20% FCS. 10 mg/ml extracts and fractions were dissolved in DMSO5%, at 10- 0.625 mg/ml and Glucantime was dissolved in PBS at 40-2.5 mg/ml. The antiamastigote activity was evaluated by the MTT method. In vivo model, 1 million stationary phase of promastigote was inoculated in the base of tail female BALB/C mice, after 8 week the lesions of L. major were created, mice randomly were distributed to 4 groups: test, placebo, glucantime and control. Mice were treated for 30 day, the diameter of lesions was measured weekly. The extract of leave, fruit and fractions of leaves had potent anti leishmanial activity, the most activity was related to leave extract. In the test group significant difference was seen, but no significant diffe Juniperus excelsa, Leishmania major, MTT CL

Keywords: leishmaniasis, Treatment, Plant

### APPLICATION OF HSP70 GENE FOR IDENTIFI-CATION OF DIFFERENT LEISHMANIA SPECIES WHICH INFECTED HUMAN IN IRAN

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Leishmania is a protozoan parasite of Kinetoplastida orders which apeares in two living forms: 1- amastigote, 2- promastigote. It causes three different type of zoonotic diseases: Cutaneous leishmaniasis, mucocutaneous leishmaniasis and visceral leishmaniasis. One of the most important diagnostic methods for determination of Leishmania parasites is review the parasite genome by molecular techniques. HSP70 gene is one of the molecular markers that is used for Leishmania diagnosis. The main objective of this project is using HSP70 Gene for identification of different Leishmania species infected human in Iran. 70 positive cutaneous (urban, rural) and visceral leishmaniasis samples were collected from Mashhad, Kashan and Meshkin Shahr cities. The DNA was extracted with phenol-chloroform and DNG-Plus kit. A 1400 bp fragment of HSP70 gene were amplified for all the samples and HaeIII enzyme was used for recognition of all PCR products. Zoonosis cutaneous leishmaniasis and visceral samples couldn't be distinguished from each other with HaeIII enzyme. So, Hinf I enzyme was used to separate VL from ZCL samples. Suspected samples were sent for sequencing. Molecular analysis (PCR-RFLP and sequencing) revealed 71.42% of samples from 28 Mashhad cutaneous samples and 53.57% of samples from 28 Kashan cutaneous samples, were identified as ACL with 99% homology with Leishmania tropica in GenBank (FN395026). 28.57% and 46.42% of samples from Mashhad and Kashan were cutaneous samples respectively and were identified as ZCL with 97% homology with Leishmania turanica in GenBank (HF586356) and all visceral samples had 99% homology with Leishmania infantum in GenBank (GQ121006).Our results show the ability of HSP70 gene in identification of different Leishmania species that cause human diseases. We also discriminate Leishmania species that cause ACL, ZCL and VL in Mashhad, Kashan and Meshkin Shahr city by using PCR-RFLP and sequencing analysis of HSP70 gene.

**Keywords:** PCR-RFLP, HSP70, multi alignment, leishmaniasis rence was observed in the placebo and glucantime group.According to our results *J. excelsa* had potent anti leishmanial activity, further research may prove it as an alternative for chemical anti leishmanial drugs.

Keywords: cutaneous leishmaniasis,





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# SEROEPIDEMIOLOGICAL SURVEY OF VISCERAL LEISHMANIASIS AMONG NOMADIC TRIBES OF SOUTH OF KERMAN PROVINCE, SOUTHEAST-ERN IRAN: AN OBSERVATIONAL STUDY FOR IMPLICATION TO HEALTH POLICY

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Visceral leishmaniasis (VL) or kala-azar is a parasitic disease caused by the species of Leishmania donovani complex. Mediterranean type of the disease is endemic in some parts of Iran and more than 95% of sero-positive cases were reported in children up to 12 years of age. A cross- sectional study was conducted to determine the seroprevalence of VL in nomadic tribe's population of the south of Kerman province. Totally, 862 blood samples were collected from children up to 12 years old from nomadic tribes of the studied area. Before sampling, a questionnaire was filled out for each case. All the collected blood samples were examined after the plasma separating by direct agglutination test (DAT) for detection of anti-Leishmania infantum antibodies. The cut-off titer of ≥ 1:3200 with specific clinical features was considered as VL. Altogether, 25 (2.6%) of the collected plasma samples showed anti- Leishmania antibodies at titers ≥1:800 and 6 of them (0.6%) showed titers  $\geq 1:3200$  with mild clinical manifestations. None of the seropositive cases had a history of kala-azar. Children of 5-8 years old showed the highest seroprevalence rate (4.1%). Also, there was not any significant difference between the rate of seropositivity in males (0.58 %) and females (0.67%), (P=0.225). Although the seroprevalence of VL is relatively low in children up to 12 years old from nomadic tribes of the studied area, due to the importance of the disease, the surveillance system should be monitored by health authorities.

Keywords: visceral leishmaniasis, seroprevalence, direct agglutination test, nomadic tribes, Iran

### PREVALENCE OF SERUM ANTIBODIES AGAINST LEISHMANIA INFANTUM IN RODENTS NORTH WEST IRAN

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Kala-azar is a visceral disease common to humans and many animals, including dogs and canids that are transmitted by sandflies.In this study some villages with the highest prevalence and incidence of visceral leishmaniasis in humans and dogs were selected. Then 180 different species of rodents were collected using live trapping. After separation, serum maintained in -20°C and were transferred to Tehran University of Medical Sciences and titration of antibody against L. infantum was done by direct agglutination and diluents with 1.78% 2-mercapto ethanol. Impression smears were prepared from the spleen and liver of rodents, stained with Giemsa and examined by light microscope. Although the cut off point is 1:320 in animal models but due to the lower sensivity of rodents to L. infantum existence of any anti-Leishmania antibodies is valuable in rodents. Three serum samples from 180 captured rodents showed 1:20 titer and two others 1:40 titer. Amastigotes were not observed in any of the slides prepared from the spleen and liver of these rodents.Rodents for being too close to human dwellings may be potential animal reservoirs for visceral leishmaniasis along with domestic and wild canids.

Keywords: visceral leishmaniasis, direct agglutination test, rodents, Iran





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#### ANTI-LEISHMANIAL ACTIVITY OF PRENYLAT-ED COUMARINS FROM FERULAGO ANGULATA AND PRANGOS ASPERULA

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Leishmaniasis causing by different numbers of Leishmania species, has a wide spectrum of signs and symptoms and causes enormous mortality and morbidity annually. There are evidences for anti leishmanial effects of prenylated coumarins. Hereby, leishmanicidal effect of some prenylated coumarins, osthole, suberosin and suberosine epoxide isolated from Prangos asperula and Ferulago angulata were assessed on Leishmania major strain (MRHO/IR/75/ER). This is the first report on pharmacological effect of suberosin epoxide. Osthol has shown a significant anti leishmanial effect on promastigotes in early hours of exposure with IC50 of 14.40  $\mu$ g/ml.IC50s of osthole and suberosin epoxide after 48 hours were 10.79 and 54.0 µg/ml, respectively. Suberosin showed no remarkable effect in these concentrations. Substantial difference between efficacies of two isomers, osthole and suberosin remarks the importance of prenyl substituent location on C-8.

Keywords: Leishmania, prenylated coumarins, suberosin epoxide, suberosin

THE PCR-RFLP-BASED DETECTION AND IDEN-TIFICATION OF THE LEISHMANIA ISOLATES CAUSING HUMAN CUTANEOUS LEISHMANIASIS IN KHORASAN-RAZAVI PROVINCE, NORTHEAST OF IRAN

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Cutaneous leishmaniasis (CL) is a widespread tropical infection which has a high incidence rate in Iran. Leishmania tropica, the causative agent of anthroponotic cutaneous leishmaniasis (ACL), and Leishmania major, which causes zoonotic cutaneous leishmaniasis (ZCL), are endemic in various parts of the country. Determining the epidemiological aspects of this disease is important for planning of control programs. The aim of this study was to evaluate the reappraisal of the diagnosis and epidemiology of CL in Khorasan-Razavi province, by different clinical, parasitological and molecular assays among patients suspected to CL during 2013-2014.DNA was isolated from 94 Giemsa-stained smears of lesions from suspected cases of cutaneous leishmaniasis and used for PCRbased diagnosis of Leishmania infection. Each smear microscopically examined and Patients' information such as age, sex and sites of ulcer (s) were registered and analyzed by Chisquare test using SPSS 16 software.Of 94 patients, 51 cases (54.3%) were male and 43 (45.7%) female. The most frequent age group was 20-29 years old (27.2%). Hands, face and feet were the most common sites of ulcers. All of 97 cases (100%) were positive by MO-ITS-PCR-RFLP. The 'Limits of Detection (LOD) for primer pairs were determined by 'Serial Dilution Assay' (SDA) based-PCR: 1-6 parasites/mL and 1 × 10 2 parasites/mL for ITS1-PCR and the method targeting ITS (MO), respectively. Characterization of Leishmania isolates collected from different parts of Khorasan-Razavi province showed that L. tropica is predominant agents of CL in Mashhad and is distributed in most endemic areas of the city. Moreover, this study revealed that ITS-PCR-RFLP based on our designed primers is a suitable method for characterization of Leishmania species.

Keywords: cutaneous leishmaniasis, ITS, LOD, Khorasan-Razavi, Iran





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### EPIDEMIOLOGICAL CHARACTERISTICS OF CU-TANEOUS LEISHMANIASIS IN PATIENTS RE-FERRED TO HEALTH CENTERS OF KASHAN (2011-2014)

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Leishmaniasis is a major health problem in Iran. Epidemiological studies are effective in disease control and preventive measures. The aim of this study was to investigate the epidemiology of cutaneous leishmaniasis in Kashan County during 2011 to 2014. All confirmed and recorded cutaneous leishmaniasis cases that were diagnosed during 2011-2014 in Kashan County entered in our study and their demographic and epidemiological information was analyzed by SPSS software. Of 1670 patients referred to kashan health centers, 1133 (68%) cases were positive. The positive cases in 2011-2014 were 168 (14.8%), 275 (24.3%), 394 (34.7%), 296 (26.2), respectively. The highest frequency of patients was in Ravand area 434 (38%). The highest incidence was in 20-29 years old group 207 (18.3%). In 793 (70%) cases the lesion was on hands and 592 (52.2%) patients had only one lesion and 541 (47.8%) had more than one lesion. The appearance of disease had the highest rate in autumn with 462 cases (40.8%). Also molecular studies showed that the dominant Leishmania species for cutaneous leishmaniasis in Kashan County were L. tropica (90%) and L. major (10%), respectively. Conclusions: Based on obtained results, as kashan city is one of important endemic area for anthroponotic cutaneous leishmaniasis in Iran, rapid screening and early treatment should be considered.

Keywords: cutaneous leishmaniasis, epidemiology, Kashan, Iran

#### PREVALENCE SURVEY OF VISCERAL LEISH-MANIASIS IN JAHROM CITY, FARS PROVINCE, IRAN

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Visceral leishmaniasis is a zoonotic disease and Fars province is one of the endemic areas. Due to the presence of visceral leishmaniasis in the Jahrom city, this study carried out to investigate the prevalence of visceral leishmaniasis in this area during 2004-2012. Information of positive visceral leishmaniasis cases which recorded in the health center of Jahrom city during 2004-2012 were collected and evaluated based on demographic characteristics of patients. During this perod, forty patients with kala-azar were recorded in Jahrom with the highest number related to the years 2004 and 2012 (9 cases). In addition, disease frequency was higher in men (57.5%) than women (42.5%). The results showed that 85% of cases were living in rural areas while 15% of them were residing in urban areas. Most of the patients had a 1/128 serum positive titration. According to the results that indicated higher frequency of visceral leishmaniasis in rural areas than urban areas, paying more attention to health education and participation of related organizations and authorities will be essential for prevention and control of this serious disease.

Keywords: prevalence, visceral leishmaniasis, Jahrom





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EPIDEMIOLOGICAL STUDY OF CUTANEOUS LEISHMANIASIS OUTBREAK IN AN ENDEMIC AREA OF KHARAMEH COUNTY OF FARS PROV-INCE IN 2014

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sity of Medical Sciences, Shiraz, Iran.

Leishmania is an obligatory intracellular protozoa which is transmitted to vertebrates by sandflies. Leishmania major and Leishmania tropica are the main causal agents of cutaneous leishmaniasis (CL) in Iran. Kharameh County, located in Fars province in southwestern Iran, is one of the most important endemic areas of CL in the country. An outbreak of disease was reported and this study was done to determine the epidemiological factors. A across-sectional study was conducted on city and 4 surrounding villages. From 277 households living in this area, 1400 participants were surveyed randomly from November to December 2014. Bio-data of cases were collected and recorded in a proper questionnaire. For identification of the causative agent, PCR was performed on 60 cases. Out of 1400 cases, 23% (328/1400) had leishmaniasis. The analysis of data indicated a high prevalence of leishmaniasis in Kharameh County. The rate of ulcers and scars was 15% and 9%, respectively. In this study, Leishmania major was identified in the majority of cases. Age, sex, lesion size and number of lesions were also investigated in this study.High prevalence of the disease in this county showed that this area encounter with a major health threatening due to low level of health facilities such as lack of safe drinking water, presence of trash around homes and schools and proximity of houses to the farms. So, comprehensive epidemiological studies for recognizing the new vectors and reservoirs hosts of the disease in this hyper endemic regionare is recommended.

Keywords: cutaneous leishmaniasis, Leishmania major, zoonoses, prevalence, PCR, Iran

### GROWTH STIMULATION EFFECT OF HUMAN URINE IN *LEISHMANIA MAJOR* PRO-MASTIGOTES

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Leishmania synthetic media can be divided into two categories: single-phase and two-phase which are widely used with fetal calf serum (FCS) and rabbit blood, respectively in mass culture and initial isolation of Leishmania species. Due to the expensive nature of FCS and also technical problems in the preparation and use of rabbit blood, the stimulatory effect of human sterile urine on the growth of L. major was investigated. Single-phase RPMI 1640 and biphasic agar culture medium supplemented with different concentrations of sterilized human urine and standard control (RPMI1640 containing 10% FCS and 10% Agar containing rabbit blood) were prepared. L. major promastigotes cultured in studied media. At specified intervals of the parasites growth and by multiply counting the average number of promastigotes reproduced in any computing environment, in compared with control media, the number of promastigotes were determined. Maximum average of L. major promastigotes in the culture with 10% human sterile urine was approximately 65.106. The average number of promastigotes in RPMI1640 medium containing different concentrations of the urine (2, 4, 6, 12, 14 and 16%) showed no difference with controls (P>0.05). Maximum density of L. major promastigotes average was approximately 21.106 in culture media supplemented with 20% urine, which showed no significant difference compared with (NNN) control medium (P>0.47). The results showed that sterile human urine, regardless of the concentration, stimulates L. major promastigote cell division and could be a good alternative for FCS and rabbit blood in parasites culture medium.

Keywords: human urine, Leishmania major, stimulatory effect





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# FIRM IDENTIFICATION OF LEISHMANIA PARA-SITE AMONG THE MAIN VECTOR FOR ZOONO-TIC CUTANEOUS LEISHMANIASIS IN ABARKOUH DISTRICT OF YAZD PROVINCE

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Leishmania major is the main causal agent of Zoonotic Cutaneous Leishmaniasis (ZCL) in Iran. Rodents of Gerbilidae family are reservoir hosts and humans, as accidental hosts, are infected through the bites of infected Phlebotomus papatasi sandflies.Since Abarkouh district of Yazd province is a focus of ZCL in Iran, we have designed this study to firmly identify the main vector of the disease through molecular techniques. Sandflies were collected using sticky paper, aspirators and CDC miniature light traps. After dissection, head and terminal part of abdomen were mounted for morphological identification and the rest was used for DNA extraction. Leishmania parasites were detected using Nested PCR, targeting ITSrDNA gene, and were confirmed after sequencing. Of 500 sandflies captured from Abarkouh district during 2010-2011, 150 were identified as female Phebotomus papatasi of which 20 were infected by Leishmania. After performing RFLP and sequencing, 15 infected Phlebotomus definitely confirmed to have L. major. According to the results of this study, L. major was definitely confirmed in P. papatasi. The 5 positive samples have not yet been identified as Leishmania major. It appears that more than one Leishmania parasite is probably circulating in Abarkouh district of Yazd.

Keywords: Leishmania major, Phlebotomus papatasi, identification, Abarkouh. PREVALENCE SURVEY OF CUTANEOUS LEISH-MANIASIS IN JAHROM CITY, FARS PROVINCE, IRAN (2013-2014)

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Jahrom is one the endemic areas for cutaneous leishmaniasis in Iran. The present study was performed to investigate the prevalence of cutaneous leishmaniasis in Jahrom during the years 2013-2014. Information of cutaneous leishmaniasis positive cases recorded in the health center of Jahrom city during 2013-2014 were collected and evaluated based on demographic characteristics of patients. The number of recorded cutaneous leishmaniasis cases was 405 in this area during the study period, including 57% males and 43% females comprising of 94.8% Iranian, 4.7% Afghan and 0.5% Iraqi. The results indicated that 45.2% of the patients were living in urban areas and 54.8% were residing in rural regions. 53.8% of cases were detected as urban (dry) cutaneous leishmaniasis and 46.2% rural (wet) cutaneous leishmaniasis. The highest frequency of ulcers was found on the hands (37.3%). The results indicated that cutaneous leishmaniasis frequency was higher in males than in females, also the frequency was more in rural areas than urban areas. Moreover, the prevalence of both types of dry and wet cutaneous Leishmaniasis nearly is equal. Therefore, preventive measures should be continued in the area

Keywords: prevalence, cutaneous leishmaniasis, Jahrom





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### EPIDEMIOLOGY OF CUTANEOUS LEISHMÁNIÁ-SIS IN SABZEVAR, IRAN, DURING 2009-2013

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Introduction & Objectives: Cutaneous leishmaniasis (CL) is still considered as an important health problem in many parts of Iran. This study was conducted to survey the epidemiological aspects of CL in Sabzevar. This study was performed on all confirmed and recorded CL diagnosed during 2009-2013. Patients data such as age, sex, habitat, numbers and sites of the ulcer (s) were recorded. Of 2158 patients, 1227 cases (56.9%) were male and 931 (43.1%) female, 346 (16%) resided in urban, 1812 (84%) lived in rural areas. The most infected age group was 20-40 years old (33.4%). Frequency of the ulcers on the body surface was: one ulcer (38.4%), two ulcers (22.9%), and three or more ulcers (35.3%). The prevalence of disease based on infected organ was: 11.3% on face, 35.8% on hands, 22.2% on foot and 30.7% in all organs. Distribiotion of diseases in 2009 was 220 cases (10.2%), in 2010, 318 cases (14.7%), in 2011, 631 cases (29.2%), in 2012, and 507 cases (23.5%) and in 2013, 482 cases (22.3%). Based on the results, it is confirmed that Sabzevar area is one of the important endemic regions for cutaneous leishmaniasis and surveillance measures for control of diseases are contiouously needed.

Keywords: epidemiology, cutaneous leishmaniasis, Sabzevar

THE EFFECT OF NO OINTMENT ON THE LE-SION CAUSED BY LEISHMANIA MAJOR IN BALB/C MICE

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Leishmaniasis is a parasitic disease caused by different species of the Genus Leishmania with distinct clinical manifestations including cutaneous, muco-cutaneous and visceral forms. Incidence rate of the disease is 1.5 to 2 million annually. Cutaneous leishmaniasis (CL) is the most common form of the disease and is endemic in various parts of Iran and presents as a public health problem. Treatment of CL is a worldwide challenging issue. Standard treatment of CL is solely depended on use of pentavalent antimony which accompanies with side effects and is not fully effective. Therefore, development of an effective and practical treatment strategy is crucial. Leishmania lives and multiplies in macrophage cell lineages. Macrophages upon activation induce a high level of NO which kills Leishmania. In this study the therapeutic effect of NO ointment on the cure of cutaneous lesion induced by L. major and the immune response generated in the infected mice was evaluated.Balb/c mice (female and male, separately) were infected with L. major in the footpad. As soon as skin lesions developed, the mice were divided in 4 groups and were treated with either glucantime, NO ointment, NO ointment with erythromycin, or Vaseline for a period of 4 weeks. The lesion size was monitored by weekly measurement of footpad swelling using a digital caliber. The immune responses of different groups was checked by spleen cell culture stimulated with Leishmania antigens, the culture supernatants were collected and IFN-y and IL-4 levels were titrated using ELIZA method. The results showed that the lesion size decreased in experimental groups. The size of lesion was significantly smaller in the treated groups than the group which received Vaseline. It is concluded that NO ointment is worth to be considered as a treatment candidate for CL.

Keywords: nitric oxide, leishmaniasis, Balb/c mice, cutaneous leishmaniasis





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#### COMPARISON OF GROWTH OF LEISHMANIA TROPICA IN THREE TYPES OF MURINE MAC-ROPHAGE

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Leishmaniasis is a major health problem in some endemic foci in different countries including Iran. Leishmania tropica is the causative agent of anthroponotic cutaneous leishmaniasis (ACL). Although control of ACL depends on treatment of the patients but available drugs are not fully effective. Studies on various aspects of L. tropica are solely done on cell culture, and so far no animal model has been developed for invivo studies. In this study, peritoneal macrophages, femour macrophages and J774 A.1 macrophages mouse cell line infected with new standard strain, IR/09/khamesipour-Mashad Leishmania tropica, and the drug effectiveness on these infected macrophages was evalauated.J774 A.1 macrophages mouse cell line, femurs and peritoneum macrophages were attached to the slide and incubated at 37°C, with 5% CO2 for 24 hr. L. tropica promastigotes in stationary phase were added to the macrophages and incubated for another 24 hrs, then different concentrations of glucantime (MA) were added and incubated for additional 72 hours. The slides were stained by Giemsa and checked under light microscope. The number of infected macrophages and the number of amastigotes per 100 macrophages were recorded. To ensure the accuracy of the results, the experiments were repeated three times. The result showed the concentration of 50µ/ml MA with J774 A.1 macrophages mouse cell line, femurs and peritoneum significantly (P<0.005) inhibitedin vitro growth of Leishmania tropica amastigote stage. Various concentrations of MA plus either J774 A.1 macrophages mouse cell line, femurs and peritoneum macrophages significantly inhibited the growth of L. tropica in same concentrations, although in J774 A.1 macrophages mouse cell line the number of infected cells and amastigotes were less than femurs and peritoneum macrophages.

Keywords: Leishmania tropica, meglumine antimoniate, macrophage, femurs, peritoneum J774 A.1

### COMBINATORY EFFECT OF MEGLUMINE AN-TIMONIATE PLUS PAROMOMYCIN, ALLOPURI-NOL OR MILTEFOSINE ON IN VITRO GROWTH OF AMASTIGOTE STAGE OF LEISHMANIA TROP-ICA

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The first-line choice treatment for leishmaniasis is still meglumine antimoniate derivatives but the efficacy of the drugs particularly against L. tropica is not acceptable and resistance is emerging. In the current study the effect of meglumine antimoniate (Glucantime, MA) combined with either paromomycin, allopurinol, or miltefosine was evaluated on L. tropica amastigote growth in in vitro model. J774 A.1 macrophage mouse cell line was attached to the slide and incubated at 37°C, with 5% CO2 for 24 h. L. tropica promastigotes were added to the macrophages and incubated for another 4 hr and then different concentrations of glucantime and paromomycin, allopurinol or miltefosine were added and incubated for additional 72 h. The slides were then dried, fixed, stained by Giemsa and checked for presence of amastigotes under a light microscope. The number of infected macrophages and the number of amastigotes per 100 macrophages were recorded. The study was performed in triplicate. The results showed that in comparison with Glucantime alone, various concentrations of MA combined with different drugs significantly inhibited in vitro growth of L. tropica amastigotes (MA plus paromomycin; p≤0.009), MA plus miltefosine;p≤0.008 and MA plus allopurinol; p≤0.010).Various concentrations of MA plus either paromomycin, allopurinol or miltefosine significantly inhibited the growth of L. tropica compared with MA alone. Further studies are required to evaluate the combinatory effect of these drugs in in vivo conditions.

Keywords: Leishmania tropica, combination therapy, macrophage, meglumine antimoniate, paromomycin, allopurinol, miltefosine.





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CUTANEOUS LEISHMANIASIS, EVALUATION OF BIOCLIMATE FACTORS DURING PAST TWENTY YEARS IN MASHHAD, (1995 – 2014)

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Leishmaniasis is an infectious disease which is caused by a protozoan parasite called Leishmania. The three clinical forms are: cutaneous, muco-cutaneous and visceral. Cutaneous leishmaniasis (CL) in turn is divided in to 3 forms as: Anthroponotic (ACL), Zoonotic (ZCL) and Disseminated (DCL). ACL usually affect the urban fringe population while ZCL is most common among the rural population. The purpose of this study was to investigate the present status of CL and the effect of Bio-climate factors on the prevalence of the disease in Mashhad during past twenty years. The raw data of confirmed cases with CL obtained from 5 health centers of Mashhad University of Medical Sciences. Environmental and metro geographical were also obtained from Meteorology office and Mashhad Municipal managing and planning organization, respectively. The collected data were analyzed using excel software and Pierson test. During past 20 years (1995 -2014), 68958 cases of CL were diagnosed at 5 health centers of Mashhad district. The results showed that the highest rate of the CL observed among the patients referred during cool seasons of each year especially on December and January. No significant relation was observed between humidity, wind speed & CL, but there was a remarkable correlation between the rates of rainfall, seasonal temperature with frequency of CL. The rate of CL was high at marginal regions of the city, where the new sensitive population live and reconstructions are always active. The highest rate of CL infection was observed during the years 2000-2001 in a focus at city center around Emam Reza shrine, where the reconstruction planning for old buildings was active at that time. Regarding the past 20 years annual histogram of CL, it is indicated that the frequency distribution of the disease corresponds with ACL form.Mashhad in an endemic city for ACL. Bio climate factors have relative influence on the rate of the disease. Most of the patients live in marginal areas of the city especially on hillsides of western area, where the new migratory and nonimmune population live with low municipal services, and reservoir nests can be observed.

Keywords: cutaneous leishmaniasis, risk factors, environmental risks, prevalence, Mashhad

# GENETIC DIVERSITY OF PLASMODIUM FALCI-PARUM MEROZOITE SURFACE PROTEIN-1 (PFMSP-1) BLOCK 2 USING NESTED-PCR IN SOUTH-EAST OF IRAN

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Merozoite surface protein 1 of Plasmodium falciparum (PfMSP1), a strong vaccine candidate against the erythrocytic stages of malaria, plays an important role in protective immunity to this disease. However, the highly polymorphic nature of this gene presents a major obstacle for effective vaccine development against malaria. In this study, genetic diversity of Plasmodium falciparum isolates from Sistan and Baluchistan province in Iran was examined using the allelic families of MSP-1 gene. From March 2011 to September 2012, 94 P. falciparum-infected blood samples were collected from malaria patients residing in four regions of Sistan and Baluchistan Province, Iran. Genomic DNA was extracted and genetic diversity of PfMSP-1 block 2 was investigated by nested polymerase chain reactions (PCR). Of 94 studied patients, 89 (94.7%) had a positive PCR outcome; the remaining five were excluded from the study. Seven different MSP-1 alleles were identified by size differences on agarose gels. MAD20 was the predominant MSP-1 allelic family identified in 46.1% of the samples, while RO33 family had the lowest frequency (7.9%). Multiple infections with two alleles were detected in 10% of the samples. The results of this study show that genetic diversity of PfMSP-1 is relatively low in south-east of Iran and most of infections are composed of one clone, which is consistent with an area of low malaria transmission. These data are useful for malaria prevention and control in Iran.

Keywords: block 2, merozoite surface protein-1, *Plasmodium falciparum*, south-east of Iran





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RETROSPECTIVE ANALYSIS OF MALARIA CASES IN MERSIN PROVINCE, TURKEY FROM 2002 TO 2011

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Malaria is an infectious disease caused by Plasmodium spp. in human in tropical and subtropical regions. In recent years, number of malaria cases has been significantly reduced because of fight with the disease in Turkey. This study aimed to investigate malaria epidemiology in Mersin province from 2002 to 2011. Over ten years a total of 303573 blood samples were taken from the people and smears were prepared. Smears were stained with Giemsa and examined under the microscope. As a result of the examination, 73 people including 44 male and 29 female were determined to be positive in terms of Plasmodium spp. It was determined that Plasmodium vivax observed in 67 cases while P. falciparum in 6 cases. Cases were mainly observed in 15 to 44 years old range, showed an increase between June-September periods and a significant decrease after 2006. Out of the 73 malaria cases, 54 cases were from Mersin province and 13 cases were imported from another province of Turkey. Six cases were transmitted from abroad. These results provide information about malaria epidemiology in an endemic area in Turkey and will contribute its prevention in Mersin province.

Keywords: malaria, epidemiology, *Plasmodium*, Mersin, Tur-key

ALLELIC VARIATION OF POLYMORPHIC VAC-CINE CANDIDATES MEROZOITE SURFACE PROTEIN-2 IN PLASMODIUM FALCIPARUM ISO-LATES FROM SOUTH-EAST OF IRAN

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Genetic diversity of Plasmodium falciparum, the main causative agent of malaria, provides the parasite with the potential of escaping the immune response and results in the selection of vaccine and drug-resistant species. Study the allelic variation of different vaccine candidate genes in regions of malaria territory could be used to design and introduce new therapeutic methods. Therefore, Merozoite surface protein 2 (MSP-2) was selected for evaluating allelic variation in the southeastern region of Iran. In this study Nested Polymerase Chain Reaction (Nested-PCR) amplification was used to determine different allelic forms of MSP-2 gene using specific oligonucleotides. A total of 94 microscopically positive P. falciparum infected individuals from south-east of Iran were included. Of all 94 Plasmodium falciparum specimens, 85 were confirmed for the presence of MSP-2 alleles. The frequency of MSP-2 different allelic classes was considerably high and calculated to be 50.5% and 34.2 % for 3D7and FC 27 respectively.Both dimorphic alleles of MSP-2 gene were detected where the frequency of 3D7 was the highest in the regions. The frequency of the alleles does not differ much from the results of studies in other regions of the world. However, this information can be beneficial to have a new vaccine designed based on studies on the candidate antigens.

**Keywords:** *Plasmodium falciparum*, merozoite surface protein 2, glutamine rich protein, allelic variation, Iran





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IMMUNOBIOCHEMICAL EVALUATION OF AN-TI-LEISHMANIAL EFFECTS OF KILLED LEISH-MANIA VACCINE (KLV) WITH BCG ADJUVANT IN BALB/C MICE INFECTED WITH LEISHMANIA MAJOR

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There are some studies applying killed Leishmania vaccines with or without adjuvant in human or Balb/c mice models to evaluate host immune responses. The aim of this study was evaluation of anti-leishmanial effects of KLV vaccine with BCG adjuvant during cutaneous leishmaniasis in order to inhibit the visceralisation of infection in Balb/c mice. The study defines its antioxidant role by induction of immune mediators such as nitric oxide (NO) in target cells, alteration of CRP pattern and designation of immunobiochemistry pathway in host. The study was carried out in naïve, control and test groups of Balb/c mice. Naïve group have not been infected with any Leishmania parasite, however control and test groups were injected with KLM vaccine plus adjuvant, there after they were challenged with promastigotes of Leishmania major. Small nodule appeared in injection site in fourth week post infection, which developed to large lesion during two months. Pathophysiological conditions including body weight, lesion size and survival rate were all evaluated during study. Finally, all of mice were killed in humane method and their spleen and liver were removed and measured for hepato/splenomegaly; amastigotes proliferation was also assessed in macrophages. No concentrations were detected in plasma and tissue suspension via Griess micro assay based on colorimetric method. Results showed that the KLV had antileishmanial activity by reducing lesion size on late infection. In KLV and BCG group, the average number of amastigotes in macrophages was lower than in other groups. Significant reductions in number of amastigotes in both spleen and lymph node were observed, indicating lower visceralization of Leishmania parasites in these target organs. No significant changes were presented in body weights, survival rates and degrees of splenomegaly in test group.It can be concluded that application of KLV and BCG had acceptable efficacy in reduction of skin lesions size and proliferation of parasites, even though a few side-effects were observed. It is indicated that KLV/BCG may have the ability to modulate host immune responses against Leishmania parasites and to reduce pathophysiology of the disease during infection.

Keywords: Leishmania major, BCG, Balb/c, killed Leishmania vaccine

# THE RATE OF PLASMODIUM VIVAX INFECTIVI-TY WITHIN GLOUCOSE-6-PHOSPHATE DEHY-DROGENASE (G6PD) DEFICIENT INDIVIDUALS IN HORMOZGAN PROVINCE, IRAN

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One of the most important enzymatic disorders that interact with malaria is G6PD (gloucose-6-phosphate dehydrogenase) deficiency. This enzyme protects red blood cells from hydrogen peroxide and other oxidative damages. Distribution of this enzyme deficiency usually accompanies with low-level distribution of malaria disease in most malarious areas. So this hypothesis may be considered that the G6PD deficiency could be protective against malaria. Totally 160 samples were taken from vivax malaria infected and non-infected individuals. Preparing blood smears and quantitative test for G6PD deficiency were employed for all of the samples. To ensure accuracy of the malaria in negative samples besides using microscopical examination, semi-nested multiplex PCR was also performed for the two groups. In microscopical examination, 36 and 124 samples were vivax malaria positive and negative respectively. Out of 36 P. vivax positive cases 3 (8.3%) cases were detected to be G6PD deficient versus 30 (24.2%) cases out of 124 P. vivax negative cases. The results showed a significant differentiation between P. vivax positive and P. vivax negative cases in the rate of G6PD deficiency (3/36 in positive cases versus 30/124 in negative cases) (P<0.05). Vivax malaria positive individuals with G6PD deficiency showed mild symptoms of malaria or even no symptom.

Keywords: malaria, Plasmodium vivax, G6PD deficiency, Iran





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#### THE ACUTE BOVINE BABESIOSIS WITH HIGH CLINICAL DISTURBANCE AND MORTALITY IN ZABOL DISTRICT, IRAN

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Bovine babesiosis is a tick-borne disease of cattle caused by the protozoan parasites of the Genus Babesia. The principal bovine Babesia species in Iran are Babesia bigemina and Babesia bovis. In southeast of Iran, Sistan area, the presence of Babesia bigemina is not confirmed yet. In November 2014 a livestock owner complained of a very acute disease in his cattle flock accompanied with fever, anemia and red urine. The Giemsa staining method was applied on peripheral blood smears. In all blood samples, heavy infection of Babesia bigemina detected. Urgent treatment with Diminazene aceturate, Oxytetracycline and anti-inflammatory drugs performed but in some cases because of progressive anemia the treatment didn't improve the disease situation and death occurred. Because of the absence of some main vectors of Babesia bigemina in the area especially Boophilus spp, the present results are very important and interesting for veterinary clinicians. We propose further investigation to identify the vectors of Babesia bigemina in Sistan region of Iran.

Keywords: bovine babesiosis, Babesia bigemina, Zabol, Iran

#### STUDY OF MALARIA IN PATIENTS REFERRED TO IMAM REZA HOSPITAL OF MASHHAD DUR-ING 13 YEARS

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Malaria is one of the most important human parasitic diseases in tropical and sub-tropical areas and is considered as one of the greatest health problems of Iran and Khorasan provinces (neighboring Afghanistan and Pakistan which cause imported Malaria) as well. The aim of this study was to document the descriptive epidemiological features of malaria in patients referred to parasitology laboratory of Imam Reza hospital of Mashhad (2001-2014). During 13 years (Mar 2001, Oct 2014) 610 patients with clinical symptoms suspected of malaria, were examined in parasitology laboratory of Emam Reza hospital of Mashhad. Thin and thick smears were prepared and examined directly under the microscope. Of 610 suspected cases of malaria 27 (4.42%) were positive in blood smear. Plasmodium vivax were seen in 18 (66.6%) patients and Plasmodium falciparumin in 9 (23.4%) patients. 85.19% of patients were male and 14.81% were female and all of them were adult. 19 patients were resident of Mashhad and 2 patients had a history of travel to Sistan and Baloochestan province and 2 of them to Afghanistan and Pakistan. Our study revealed that dominant species was Plasmodium vivax and males were affected more frequently than females. 22.2% of our patients had a history of traveling to infected area. Regarding to the multiplicity of traveling to infected neighbor countries, medical managers and practitioners must notice this disease at the border line of countries for prevention and control of imported malaria.

Keywords: malaria, plasmodium, epidemiology, Mashhad, Iran





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# HIGH-LEVEL EXPRESSION OF IMMUNOGENIC RECOMBINANT PLASMODIUM VIVAX MEROZO-ITE SURFACE PROTEIN (PVMSP-1 42 KDA) IN PGEX 6P1 VECTOR

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Detection of Plasmodium vivax specific antibodies with serological tests could be a valuable device for epidemiological researches. Whereas Plasmodium vivax cannot be simply acquired in vitro, serological tests using total or semi-purified antigens are infrequently used. Given this restriction, we evaluated whether recombinant Plasmodium vivax merozoite surface protein 1 (PvMSP-1 42 kDa) could be useful for detection antibodies from Plasmodium vivax infected person sera by serological tests. Parasite DNA was extracted from blood sample of an Iranian Plasmodium vivax-infected patient. The region of PvMSP-142 kDa was amplified by PCR, cloned into pTZ57R/T vector and sequenced. The insert was sub cloned into pGEX 6P1 expression vector. Afterwards, it was transformed into E. coli BL21 and cultured massively. Sub cloning of gene was confirmed by PCR and enzyme digestion and sequencing finally. Production of recombinant protein confirmed by SDS-PAGE. Western blot was performed by human sera to appraisal binding ability to the IgG antibodies of Plasmodium vivax infected patients. Recombinant protein was purified and estimated by Bradford assay. The specificity values of the Western blot determined with 10 sera from naturally infected individuals, 10 sera from healthy individuals and 7 sera from individuals with other infectious diseases.Our study indicated that for the Iranian population, using a western blot assay for MSP-142 recombinant protein can be used as the foundation for promotion of serological assay for the detection of P. vivax malaria.

Keywords: *Plasmodium vivax*, recombinant PvMSP-1 42 kDa, expression vector, Iran

# NEW PERSPECTIVES FOR MALARIA VACCINE DEVELOPMENT

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Malaria is caused by various species of *Plasmodium* parasites. The disease leads to huge loss of life and property in human societies. In some cases, malaria treatment is not possible. This situation has encouraged scientists to focus on malaria vaccine developments. Paying attention to the life cycle of the malaria parasite can guide the process of vaccine development. Since the parasite passes through several stages and there are interruptions in malaria transmission, many people lose their acquired immunity against the disease and even adults may reach the immunity status of infants. On the other hand, because Plasmodium parasite has different strains and antigens, the chances of achieving a vaccine that can create lasting immunity when used once is very weak. The first stage is to produce malaria vaccine based on identifying antigens, which stimulate sustainable protective immune response. The second stage involves antibody genes encoding. To be able to attack the red blood cells, Plasmodium falciparum relies on a single receptor called Basigin, which is located on the surface of these cells. The parasite binds to proteins called RH5antigens on this receptor and in fact pave the way for its entry into the red blood cell. Some vaccines, such as different types of SPF66 affect this receptor and prevent the parasite from entering into the red blood cells. This achievement has created an avenue of hope for researchers. However, malaria has caused human distress. In such an eventuality, paying attention to methods of prevention and drug treatment is inevitable.

Keywords: perspectives, malaria, vaccine, Plasmodium.





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# NEW METHODS FOR DIAGNOSIS OF MALARIA IN MALARIA-PRONE AREAS

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Malaria is still considered a problem in many parts of Africa and Middle East. A feature of the disease that has made it a health problem is its diagnostic problems. Achieving an accurate and efficient, yet affordable and cheap, method will reduce malaria risks. Taking a blood Smear from the person suspected of having the disease and its microscopic examination after staining with Wright-Giemsa stain, is the simplest and most accurate laboratory test for diagnosing the disease and determining the Plasmodium type. This is thus the most widely used test. In cases where the number of parasites in the blood is low or the patient is receiving a drug therapy, blood smear method is not effective. In addition, in evaluation of methods for controlling and eradicating Malaria where carriers of Plasmodium are followed up, blood smear method will not be accurate and serological methods should be used. In particular, indirect haemagglutination and fluorescent antibody methods are mostly used in malaria epidemiology and control studies. For diagnosis of Malaria in early stages in people recently infected with clinical signs of the disease, serological methods are not needed because the parasite is found in blood samples. In addition, in people who have repeated malaria infections or live in malaria-endemic areas, positive serology test does not indicate the infection. In malaria-prone areas, presence of some parasites like Babesia in peripheral blood smears may be diagnosed incorrectly as Plasmodium falciparum. Today, scientists use light magnet technology (MOT) where magnetic properties are used for detection of Haemozoin, a residue of the malaria parasite in the blood. This new method is preferred to previous methods for malaria testing because of more accurate identification of samples containing little parasite. Using the method, practitioners can diagnose malaria in less than a minute.

Keywords: diagnosis, malaria

Malaria is still known as a parasitic devastating global health problem. The causative agent is an obligate intraerythrocytic protozoon which belongs to the genus Plasmodium. Based on the National Strategy Plan for Malaria Control, Iran has been classified into four strata and northern parts of the country including Mazandaran, Guilan and Golestan Provinces are considered areas where the imported cases are found and the potential risk of malaria transmission still exists. The objective of this case report was to inform colleagues and authorities of the existence of this dreadful disease and also the probability of misdiagnose in north of Iran. Case history: We report a case of malaria infection in a 42-year-old woman in rural area of Mahmodabad, Mazandaran province, north of Iran. She was complaining about recurrent fevers, sweating, headache and myalgia in back. After her first admission to hospital, due to misdiagnose she did not receive proper treatment and the patient suffered from clinical manifestations again. Eventually in the second admission to another hospital, after a precise examination on her thick and thin blood smear the agent of disease was recognized appropriately as *Plasmodium vivax* and treated accordingly. This point is noteworthy to mention that malaria initial clinical manifestations are similar to flu-like signs and can resemble other conditions such as gastroenteritis, septicemia and viral diseases. Hence, this point may lead to misdiagnose of patients such as this report at first admission.

Keywords: malaria, *Plasmodium vivax*, infection, tropical diseases, Iran.





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### MOLECULAR SURVEILLANCE OF PLASMODIUM VIVAX AND PLASMODIUM FALCIPARUM DHFR MUTATIONS IN ISOLATES FROM SOUTHERN IRAN

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In Iran, both Plasmodium vivax and P. falciparum malaria have been detected, but P. vivax is the predominant species. Point mutations in dihydrofolate reductase (dhfr) gene in both Plasmodia are the major mechanism of pyrimethamine resistance. Understanding the epidemiology of drug resistance is necessary for effective drug policy. From April 2007 to June 2009, 134 blood samples were collected from P. vivax and P. falciparum malaria patients in two endemic areas of southern Iran for pvdhfr and pfdhfr using various PCRbased methods. The majority of isolates (72.9%) showed the wild type amino acids at five codons (33, 57, 58, 117 and 173) of pvdhfr. Amongst mutant isolates, the most common pvdhfr alleles were double mutant with combination of two mutations, S58R-S117N or F57L-S58R. Mutations at residues 33 and 173 were not detected. Triple mutation in 57L/58R/117N is the first report in pvdhfr gene of Iranian P.vivax isolates. All P. falciparum samples analysed (n =16) possessed a double mutant pfdhfr allele (59R/108N) while retaining a wild-type mutation at position 51. All of allelic types of pvdhfr (7 types) and pfdhfr (1 type) were represented in GenBank (accession number AB547452 AB547459). This study has determined low molecular prevalence of resistance to pyrimethamine in P. vivax and a high level of resistance in P. falciparum because the drug was mainly given to treat falciparum malaria. However its use as a firstline antimalarial for P. falciparum should be discouraged but mutant haplotypes in P. vivax, have not yet reached an alarming threshold. These data reinforce the importance of performing molecular surveillance by continuous chemo resistance assessment.

**Keywords:** *Plasmodium vivax*, *Plasmodium falciparum*, pyrimethamine, point mutations, drug resistance

# THE EFFECT OF HYDROALCOHOLIC EXTRACT OF NIGELLA SATIVA ON INFECTED MICE WITH PLASMODIUM BERGHEI, EVALUATION OF IM-MUNE DEVIATION AND THE SERUM LEVEL OF IFN-Γ&IL-4

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Introduction 7 Objectives: Malaria is one of the most widespread infectious diseases of tropical countries with an estimated 207 million cases globally. Treatment of malaria has become more difficult because of drug-resistant parasites. Therefore, safe and effective new drugs are needed. Traditional medicine is an important source for new drugs. Because of its simplicity, lower cost, low rate of serious complications, and greater tolerability, there has been a great progress in using herbal medicines for treating diseases. The aim of this study was to evaluate the anti-malaria and immune modulatory effects of Nigella sativa against Plasmodium Berghei in vivo. The powder was macerated in methanol, filtered with Bokhner hopper and solvent was separated in rotary evaporator. The toxicity of herbal extract was assessed on naive mice with high, average and low doses. Antimalarial efficacy and cytokines level of IFNg and IL-4 was investigated on five groups of Plasmodium Berghei infected Balb/c mice. Percentage of parasitaemia and surveillance were also evaluated. The results of this study showed no toxicity even by high concentration of herbal extract. A significant reduction in percentage of parasitaemia was observed in treatment group. Infected mice that has been treated with Nigella sativa have a significant increment in serum level of IFNg but not for IL-4. Treated mice have higher surveillance. Nigella sativa extracts showed antimalarial effects against murine malaria with some efficacies on increasing surveillance. The immuneregulation effects of treating Plasmodium Berghei in mice enlighten a new treatment for brain malaria in human. However, it is necessary to evaluate the immune mechanism and also find the major component of this herbal extract by further studies.

Keywords: Nigella sativa, Plasmodium Berghei, anti-malaria, immunomodulator, in vivo





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PARASITOLOGICAL AND MOLECULAR INVES-TIGATION OF BABESIA MICROTI IN RODENTS OF SARAB DISTRICT OF EAST AZERBAIJAN

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The cause of babesiosis, piroplasmosis or red water fever is a small protozoon called Babesia. Vast majority of animals including; aquatics, amphibians, rodents, reptiles, birds, mammals and human beings are infected with this parasite. Thus, it is considered one of the most important diseases of zoonotic parasitic infections and is introduced as a health problem in some countries of tropical areas, which at the same time, is of economical importance. The cause of babesiosis in Iran is Babesia microti and rodents are one of the reservoirs of the disease parasite. This investigation is the first molecular study for determining rodents as reservoir hosts of Babesia microti in Sarab city and surrounding villages. First of all, 100 rodents of 4 different species consisting Mus musculus, Meriones persicus, Cricetulus migratorius and Mesocricetus auratus from different villages of Sarab city were hunted using live traps. Then blood samples of all hunted rodents were tested by PCR and parasitologic studies (preparing impression smear of spleen tissue and thin peripheral blood smear) were carried out. By dissection and parasitological studies of all the rodents of 4 different species, in 3 of them which were mice, no schizont was seen in impression smear of spleen tissue but pear shaped intra erythrocytic Babesia parasite was present in thin blood smear study. Finally, parasitologic test results were confirmed using molecular method of PCR. The isolated parasite genus was determined to be Babesia microti. Conclusions: It was specified in this study that rodents, especially mice could be as reservoir hosts for babesiosis in Sarab city of East Azerbaijan province of Iran.

Keywords: Babesia microti, rodents, Iran, PCR

IMPORTED MALARIA IN HASHTGERD, IRAN, 2010-2014

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Malaria is endemic in more than 105 countries and a communicable disease transmitted to humans through the bite of a female Anopheles mosquito. The infection is still one of the most important public health problems with more than 200 million cases and 655,000 deaths every year. Although many countries of the world have been declared malaria free, it is estimated that the total number of travelers that may acquire the infection from these endemic areas may be as high as 30,000 individuals per annum. In this retrospective study, 14 cases were recorded in the health centre of the city of Hashtgerd during 2010 to 2014. The statistical indices were calculated using SPSS software. In this study of 14 patients, 13 were male and 1 female. In the imported cases, 2 Plasmodium species were identified: P. falciparum (n = 2 [14%]), P. vivax (n = 10 [71%]), and mixed infections (n = 2 [14%]). In this analysis, an imported case of malaria was defined as case of malaria acquired in a known malarious area outside Hashtgerd. Therefore, Prevention of malaria requires educating travelers about the health risks associated with travel and the need to obtain pretravel medical advice, and educating health-care providers regarding optimal and accurate malaria prevention recommendations.

Keywords: imported malaria, Hashtgerd, P. falciparum, P. vivax





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A SERO-EPIDEMIOLOGICAL STUDY OF VIVAX MALARIA IN CHILDREN UNDER 9 YEARS OLD BY IFA METHOD AND DETECTING POSITIVE CASES USING SEMI-NESTED MULTIPLEX-PCR IN MINAB AND JASK DISTRICTS OF HOR-MOZGAN PROVINCE, IRAN

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Introduction & Objjectives: Malaria is a protozoan disease, which happens as a result of being affected by Plasmodium falciparum, P. ovale, P. vivax, P. malariae, P. knowlesi. According to WHO documents in 2012 about 207 million cases with 627000 death were reported from malariaous area in the world. Although microscopical examination is the gold standard method for diagnosing malaria parasites, seroepidemiological study is a selective method for detecting antiplasmodial antibodies. Results of the method can facilitate to implement malaria elimination programs. The main aim of malaria elimination program is to interrupt local autochthonous transmissions of the disease and if positive cases are being found, they supposed to be imported cases. The pourpose of this study is to assess anti malaria antibody in children less than 9 years old using IFA test method. This research can provide information about if there is any local transmission of P. vivax malaria in the areas. A semi nested multiplex PCR was employed if there is found any positive case. Three hundred eighty samples were randomaly collected and Giemsa stained thin and thick blood smears were prepared for parasitological study. Moreover, some of the blood samples were collected in micro-hematocrit tubes for IFA test and two drops of blood put on DNA Banking Card for PCR studies.Results of this study showed that there was no detected positive case, neither in microscopical examination nor in IFA test. Namely, there was not any active malaria transmission in the studied districts.It could be concluded that, malaria elimination program is successfully in progress at the districts.

DISTRIBUTION OF BABESIA INFECTION IN LIVESTOCK REFERRED TO VETERINARY CLIN-ICS IN ZABOL, SOUTHEAST OF IRAN

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Babesiosis is one of the most prevalent parasitic diseases in animals and rarely in humans, which causes great economic losses in the livestock. Due to precence of numerous streams and pastures, animal husbandry is thriving in this region. The objective of this study was to determine the distribution of babesiosis in animals referred to veterinary clinics in the Zabol district in 2014. This study was performed as a crosssectional descriptive survey. The blood samples were taken from marginal ear of 472 animals consisting of 280 sheep, 101 goats and 91 cattle. A questionnaire was completed for each animal, recording sex and age. Thin and thick blood smears were prepared. Blood smears were fixed with methanol, stained by Giemsa staining, and studied under a light microscope. Finally, data were analyzed using SPSS 20.0 and Chi-square test. P<0.05 was considered significant. The results showed that 44 sheep (16.8%), 21 goats (20.2%) and 19 cattle (19.7 %) were infected with Babesia parasites. No significant differences was found between the age and sex of animals with Babesia infection. The results of this study showed that babesiosis is highly prevalent among animals in Zabol city. It appears that appropriate ecological conditions for presence and spreading of Babesia infected ticks can be a major cause for onset of the disease in this area. Further studies on the various aspects of the disease, especially in areas where little research has been done, is needed for establishing the prevention and control programs of babesiosis Zabol.

Keywords: Babesiosis, distibution, livestock, Zabol

Keywords: malaria, IFA, serology, Hormozgan, Iran





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# DIFFICULT TO BELIEVE THAT "PLASMODIUM VIVAX" IS BENIGN: A PRELIMINARY STUDY

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Plasmodium vivax is considered as a human pathogen that usually cause benign tertian malaria, but nowadays it is deemed an agent with complications and mild hematological abnormalities. Thrombocytopenia is one of the most important disorders ocurring in patients infected with Plasmodium vivax. This study aimed to determine the frequency of thrombocytopenia in patients infected with P. vivax. Thirty-seven vivax malaria patients were selected for this study. The infection confirmed via microscopical examination using Geimsa staining. Platelet count was done with a manual method for samples and results were analyzed in compare with healthy individuals. This study was conducted at Tehran University of Medical Sciences in October 2014.A total of 37 patients were included in this study. The results showed that eleven cases (29.7%) had thrombocytopenia (<140,000 platelets/µl). One vivax malaria infected case encountered with acute hemorrhage with platelet count of below 50,000 that after treating with standard antimalarial drugs the problem was resolved.In the present study thrombocytopenia was clearly identified in patients with vivax malaria infection. So it is important to ensure that the disease receives special care or treatment. Severity of P. vivax should be considered and further researches would be needed in the etiopathogenesis and treatment.

Keywords: Plasmodium vivax, thrombocytopenia, severe malaria

# IMMUNOBIOCHEMICAL STUDY OF HOST MA-LARIA DISEASE BY MANIPULATION OF THE IMMUNE SYSTEM VIA NITRIC OXIDE AND MI-CROELEMENTS PATHWAYS

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Malaria is a parasitic disease which is common in tropical and subtropical regions of the world. Lipopolysaccharide (LPS) as an inducer and Dexamethasone (DXM) as an inhibitor of the immune system are used in this study. They are investigated for their effects on the amount of nitric oxide (NO) on plasma, liver and spleen, microelements including copper and zinc in plasma, hepatomegaly, splenomegaly, survival rate and the degree of parasitaemia on malaria infected mice. In this study, 24 NMRI mice were randomly divided into 4 groups of 6 animals. Entire mice were infected by the murine malaria parasite (Plasmodium Berghei). Two groups of mice were selected as controls, injected with intraperitoneal (ip) injection of normal saline. The two DXM and LPS groups of mice received concentrations of 4 and 1 mg/kg treatment respectively by 8 times ip injection every other day. Their body weight, survival rates and parasitaemia were monitored during the study. Finally, mice were euthanized by terminal anesthesia and cardiac puncture and the entire liver and spleen were removed for hepatosplenomegaly. Plasma, liver and spleen suspensions were assessed for immunobiochemical alterations including NO levels and microelements (Cu, Zn) values. The results indicated an increase in hepatomegaly of test group compared to control mice. LPS mice represented splenomegaly more than DXM one. NO in plasma, liver and spleen in both DXM and LPS group was changed, however, the only significant difference observed in the liver of LPS. The values of Zn and Cu in plasma increased in both LPS and DXM with a statistically significant difference. Moreover, DXM elevated the Cu /Zn ratio significantly in plasma of the test group mice. It is concluded that LPS and DXM can be applied as a standard and medically approved inducer and inhibitor of the immune system respectively, for experimental immunomodulation studies in animal models. In this study, LPS induced and DXM reduced immune responses in mice during malaria infection by alterations and manipulation of immunobiochemical factors via NO and microelements pathways. This may lead to an immunotherapy trial of malaria by controling the pathophysiology and degree of parasitaemia in mouse model.

Keywords: malaria, lipopolysaccharide, dexamethasone, microelements, nitric oxide





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# MOLECULAR DETECTION OF BOVINE BABESIA SPP. AND THEILERIA SPP. IN NORTH OF IRAN

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Babesiosis and theileriosis are the main fatal protozoal diseases among livestock in endemic regions such as Iran. The aim of this study was to detect Babesia and Theileria infections using microscopic and molecular methods among cattle in Mazandaran and Golestan provinces, northern Iran. We collected two hundred thin and thick blood smears (known as positive smears) from private veterinary laboratory archive of different districts in both provinces during 2012 - 2013 to confirm babesiosis and theileriosis using Geimsa staining and also polymerase chain reaction (PCR) methods with standard and species specific PCR for Babesia spp. and Theileria spp. Out of a total of 200 blood smears from Mazandaran and Golestan province, 23 cases (11.5%) were positive with Theileria using standard PCR. However, none of them were positive for Babesia spp. using standard and species specific PCR. The relatively high prevalence of Theileria infection in cattle shows the epizootic stability status of bovine theileriosis in the investigated areas. Our data showed bovine babesiosis is not common in the areas, and thus babesiosis will not pose a risk for inhabitants (mainly farmers) in these regions.

Keywords: cattle, babesiosis, theileriosis, Mazandaran, Golestan

GENETIC DIVERSITY OF RETICULOCYTE BIND-ING PROTEIN GENE IN VIVAX MALARIA PA-TIENTS IN SISTAN AND BALUCHESTAN PROV-INCE

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Malaria is one of the most important parasitic diseases in Iran. Plasmodium vivax is the predominant causative species of malaria with about 90% of the total annual reported cases of malaria in Iran. Plasmodium vivax reticulocyte binding protein 2 is a promising candidate of vaccine against malaria. The aim of this study was to determine genetic diversity of Plasmodium vivax reticulocyte binding protein 2 in symptomatic malaria patients in Sistan and Baluchistan province. A total of 50 blood samples from symptomatic patients with Plasmodium vivax were collected. DNA extraction was performed using the Qiagen kit. The RBP-2 was amplified by Nested PCR. To determine the level of pvrbp2 polymorphism, RFLP analysis was carried out using two restriction enzymes ApoI and AluI. The products were analyzed on 1.5% agarose gel. A total of 50 P. vivax samples showed single clear amplification of ~2.0 kb fragment size and none of the PCR fragments revealed size variation. From RFLP analysis, it is clear that ApoI with three distinct digestion patterns is identifying larger extent of genetic polymorphism compared to AluI. RFLP analysis demonstrated mainly two distinct digestion patterns in field isolates. This study is the first to investigate genetic diversity of PvRBP2 gene in Iranian Plasmodium vivax isolates. This information could be taken in to accounts for vaccine development.

Keywords: *Plasmodium vivax*, reticulocyte binding protein-2, Nested PCR, RFLP, Iran





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IMMUNOTHERAPY AND ANTI-MALARIAL EF-FECTS OF L-ARGININE AS PRECURSOR OF NI-TRIC OXIDE PRODUCTION TO INDUCE IM-MUNITY IN OUTBRED NMRI MICE INFECTED WITH MURINE MALARIA

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Malaria is caused by the *Plasmodium* parasites and annually kills about 800,000 people especially Africans. In patients with malaria, nitric oxide (NO) is one of the major mediators of the host immune system, produced by macrophages during the innate immune response. L-arginine (L-arg) plays a role in many metabolic pathways such as NO and parasitic infections can trigger the release of NO and other immune molecules in the body. The aim of this study was to evaluate the role of anti-malarial effects of L-arg in mouse model. Therefore, the impact of L-arg as a precursor of NO to induce immunity system in outbred NMRI mice infected with Plasmodium Berghei has been investigated. In this study, NMRI mice were randomly divided into 2 groups both infected with P. berghei including control and test groups. Test group received L-arg treatment at concentration of 0.02g/ml by intraperitoneally (ip) over 6 stages. To synchronize conditions of the two groups, control mice were injected with ip injection of distilled water (DW). Their body weight, survival rates and parasitaemia were monitored during the study. Finally, mice were euthanized by terminal anesthesia and cardiac puncture and the entire liver and spleen were removed for hepatosplenomegaly. Plasma, liver and spleen suspensions were assessed for immunobiochemical alterations including NO levels and microelements values. The obtained results were statistically analyzed by Graph Pad Prism statistical software. The degree of parasitemia decreased in test group in comparison with the control mice, however, by cease of the L-arg treatment, the parasitemia increased in this group. Therefore, it can be concluded that the reduction of parasitemia has a potent association with L-Arg injection and NO production, but the difference was not statistically reasonable (P = 0.07). Regarding the effects of L-arg injection on parasitemia in infected mice, it is anticipated that with repeated injections, a reduction in the number of parasites can be achieved. This will be a confirmation of L-arg effect on NO levels as inducer of immune function in this mouse model. This is the first practical study of malaria immunotherapy via NO pathway in animal model in Iran.

Keywords: immunotherapy, malaria, parasitemia, l-arginine, nitric oxide

DETECTION OF INFECTION RATE, INTERME-DIATE HOST AND MOLECULAR DIAGNOSIS OF THEILERIA LESTOQUARDI IN GOAT IN WEST-AZARBIJAN-IRAN

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*Theileria lestoquardi* -a protozoan belong to the order piroplasmida- cause disease in sheep and goat. The aim of this study was to evaluate infection rate, intermediate host and molecular diagnosis of *T. lestoquardi* in goat in West-Azarbijan. 400 blood samples were investigate via microscopic examination and semi-nested PCR. Also 315 tick samples collected from body surface of animal were diagnosed. The infection rate by microscopic and molecular route were 3.5% (14 samples) and 6.25% (25 samples) respectively. Also 18.4% ticks (58) were infected with *T. lestoquardi*. *Rhipicephalus* spp., *Hyalomma* spp. and *Dermacentor* spp. were identified. In semi-nested PCR a 235bp band confirmed an infection with *T. lestoquardi*.Despite a low infection rate, planning of prevention and control program is recommended.

Keywords: T. lestoquardi, theileriosis, goat, molecular diagnosis





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RELATION BETWEEN PLASMODIUM VIVAX AMA-1 VACCINE CANDIDATE GENE DIVERSITY AND AGE GROUP PATIENTS IN SOUTHEAST OF IRAN

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Apical membrane antigen-1 (AMA-1) is a promising bloodstage malaria vaccine candidate. Antibodies against AMA-1 have been detected in populations exposed to natural human infections. The introduced antibodies can be protective against malaria parasites. This protein contains an ectodomain divided in three subdomains (DI, DII and DIII) by eight disulfide bonds. Single Nucleotide Polymorphisms (SNPs) are identified in certain regions of the gene that may serve as a useful molecular marker for typing parasite populations. These markers can also provide the basic information for strategic development of mono- or polyvalent vaccine. In this study we analyzed the associations between polymorphic amino acid residues at PvAMA-1 and different age groups. The Plasmodium vivax isolates were collected from two different areas of tropical malaria endemic regions in southern and southeastern Iran during 2009-2011. Genomic DNA was extracted from the infected blood samples and then amplified by Nested-PCR and sequenced. Based on the sequencing data, a 911bp region of the ama-1 gene including the nucleotides 289 to 1199 was readable. This nucleotide sequence codes for amino acids 97 to 399 containing the major sequence of DI, complete DII and partial DIII of AMA-1. Nucleotide mutations were found in first, second and third codon positions (15, 14, and 20, respectively). Among 30 variable amino acid residues, 23 of them were dimorphic, 6 were trimorphic and the position 112 showed tetramorphic. The positions 112, 189, 190, 228, 382, 384 and 385 at DI and DII of the PvAMA-1 included substitutions with more than 2 possible amino acid residues. The distribution of polymorphic amino acid residues by age categories indicated a strong age dependence between the frequency of Q and P residues in position 384 and clinical disease in 30-40 age group also a parallel situation was found in P384, D107, K189, K190, L384 positions in 40 and more years old. Present study provides necessary information to design a malaria vaccine to be effective in different age categories and the knowledge of the sequences of the AMA-1 gene observed in the population to detect which of the variants induced the protective immunity, could help to design an AMA-1-based vaccine with a small number antigen variants in that to be effective against most variants of AMA-1.

Keywords: *Plasmodium vivax*, malaria, apical membrane antigen-1, vaccine

# PREDICTION OF INSIDE AND OUTSIDE TRANSMEMEBRANE PARTS AND COMPARISON OF PHYSICOCHEMICAL CHARACTERS OF MSP1 PROTEIN OF PLASMODIUM FALCIPARUM IN TWO ISOLATES OF MALAYSIA AND GHANA

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Plasmodium falciparum is the deadliest of five human malaria species and responsible for the majority of malaria related deaths. It is transmitted by the female Anopheles mosquito. As of 2006, an estimated 247 million human malarial infections (98% in Africa, 70% being 5 years or younger) occurred. It is much more prevalent in sub-Saharan Africa than in many other regions of the world; in most African countries, over 75% of cases were due to P. falciparum, whereas in most other countries with malaria transmission, other less virulent plasmodial species predominate. Msp1 protein sequence of Malaysia and Ghana isolates were retrieved from uniprot. Then these proteins were studied according to outside and inside transmemebrane regions as well as physicochemical characters by TMHMM server and Expasy: protparam tool, respectively. The prediction of outside and inside transmemebrane regions of mps1 in Ghana with 1682 aminoacids showed: from 1 to 1657 was outside, from 1658 to 1680 was TM and 1681 to 1682 was inside region. In Malaysia isolate from 1 to 1701 was outside, 1702 to 1724 was TM and from 1725 to 1726 was inside region in merozoite level. Two isolates had equal number of amino acids but different molecular weight. The P19598 from Ghana and the P04934 from Malaysia had 192463.4 and 196198.2 molecular weight, respectively. Estimated half-life in mammalian reticulocytes, in vitro was 30 hours for both proteins. Our study demonstrated that two proteins are classified as stable protein. The instability index for P19598 was 36.57, while P04934 showed 38.24. Finally aliphatic index and grand average of hydropathicity (GRA-VY) were different in both isolates. Studying of proteins and other properties may be useful and practical to make drugs and vaccines.

Keywords: Plasmodium falciparum, msp1, malaria, transmemebrane





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MALARIA EPIDEMIOLOGICAL SITUATION IN ZAHEDAN UNIVERSITY OF MEDICAL SCIENCES (2002-2011)

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Malaria is one the most important health problems in tropical and semi- tropical countries. Now it is one of the most important parasitical diseases in Iran, which its indigenous cases are only in the South and South-East of the country. Malaria epidemiological study at Zahedan University of Medical Sciences with the highest incidence will help researchers and planners for its elimination. In this study, 10 years necessary information was obtained from existing documents in Sistan and Baluchistan province health center and analyzed by SPSS software. In 2002, total malaria cases were 6932 whereas it was 2380 cases in 2011. In 2004, API was 9.2 (Per 1000 population) but generally this index decreased from 4.1 in 2002 to 1.1 in 2011. Although, ABER from 2002-2008 increased (from 11.2 to 19.2), at the end of the study reduced to 2.6%. Also SPR declined from 3.7% in 2002 to 1.7% in 2011. High incidence of malaria in Zahedan and having common border with Afghanistan and Pakistan indicates the importance of this parasitic disease but the decreasing trend of malaria cases in the recent years may indicate successful efforts of health system to achieve the goal of malaria elimination program in 1404.

Keywords: epidemiology, malaria, Sistan and Baluchestan, Iran

PREVALENCE SURVEY OF MALARIA DISEASE IN JAHROM CITY DURING 1388-1393

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Malaria is one of the important diseases in tropical and subtropical areas causing death of 1.5 up to 2.7 million patients in the world annually. The purpose of the present study was to investigate malaria prevalence in Jahrom city as one of the tropical areas in Iran. Information of malaria positive cases recorded in the health center of Jahrom city during 6 years (2009-2014) were collected and evaluated based on demographic characteristics of the patients. During 2009-2014, 57 malaria cases were detected in Jahrom city that among them 89.5% had a history of travel to endemic areas. The highest number of malaria cases were recorded in the year 2011 (15 cases). The results showed that 66.7% of patients were Afghan, 29.8% Iranian and 3.5% Pakistani. 35.1% of patients suffered from joint pain in addition to fever, chills and sweating. Also 57.9% of cases were living in rural and 42.1% in urban areas. Infection with Plasmodium vivax accounted for 96.8% of cases while P. falciparum was 3.5%. Among all of the patients, 38.6% cases had a history of malaria disease. The results indicated the majority of patients had a history of travel to endemic areas and in other words, the prevalence is not endemic in Jahrom region. Considering the high rate of infection of Afghan migrants, screening and preventive measures will be essential.

Keywords: prevalence, malaria, Jahrom, Iran





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# A SIMPLE, EFFICIENT AND INEXPENSIVE METHOD FOR MALARIA PARASITES' DNA CATCHING FROM FIXED GIEMSA-STAINED BLOOD SLIDES

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As parasitological or microscopic method is the gold standard and the best method for diagnosis of malaria, so fixed Geimsa-stained blood slides in the form of thick and thin blood smears are the most important data collections means of malaria, especially historical slides. The parasites are dead but their DNA is valuable for many molecular biologic researches. A simple and efficient method for catching and extraction malaria parasites' DNA with a desired yield from dried and stained blood on slides is the first and major step. Introduction of an applicable, efficient and inexpensive DNA catching method and assessment of its performance in following molecular applications was the main objective of present study.

Keywords: malaria, Plasmodium *falciparum*, Plasmodium *vi*vax, DNA extraction, fixed Giemsa-stained blood slide

#### MORPHOLOGICAL IDENTIFICATION OF ANOPHELES SPECIES IN SELECTED MALARI-OUS AREAS OF AFGHANISTAN

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Malaria is the most important vector-borne infectious disease in the world, caused by the protozoan Plasmodium spp. and transmitted by infected female Anopheles spp. Vectorial capacity differs from one species to another, even at population level and from place to place. The first step in any control program is vector species identification. Moreover, our malaria endemic areas are close to Afghanistan and Pakistan, urging extend our knowledge on malaria epidemiology (plasmodium, human and mosquito hosts) to the neighboring countries as well. Accordingly, the present study has been designed to provide baseline data on the composition of Anopheles species within selected areas of Afghanistan, hopefully assisting the authorities of malaria control program in neighboring countries and planning the joint activities against border malaria. Adult Anopheles spp. were collected through WHO/EMRO coordination from four provinces of Afghanistan (Badakhshan, Herat, Kunduz, Nangarhar), followed by morphological identification using a newly developed key to Iranian, Afghani and Pakistani Anopheles species in MVRG, PII. Out of 400 identified Anopheles specimens, about 180 (45%) were Anopheles superpictus, 110 (27%) A. stephensi and 110 (27%) A. hyrcanus. Most prevalent species in Badakhshan and Kunduz were A. superpictus, whereas A. stephensi and A. hyrcanus were found mostly in Nangarhar and Heart, respectively.Based on the out coming results, three different species were identified among samples with A. superpictus as the most prevalent, and it should be mentioned that A. superpictus & A. stephensi were considered as main vectors of malaria in their distribution areas. Having update information on the composition of Anopheles species could be useful for any future studies such as potential Plasmodium infection surveys which is on process by the authors.

Keywords: Anopheles spp, morphological identification, Afghanistan





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CORRELATION BETWEEN PARASITEMIA RATE, SIALIC ACIDS LEVEL AND INNATE AND AC-QUIRED IMMUNITY IN BUFFALOES NATURAL-LY INFECTED WITH THEILERIA ANNULATA

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Theileriosis is a tick-borne diseases which is responsible for considerable economic losses to cattle and buffalo farming in Iran. This study was done to investigate the effect of severity of Theileria annulata parasitemia on the sialic acids levels and potential of immune responses of naturally infected buffalo. Infected animals comprised 32 naturally infected buffalo with T. annulata, divided into four subgroups based on parasitemia rates diagnosed by PCR. Then clinically healthy buffaloes were also sampled as a control group. The concentrations of total sialic acid (TSA), lipid-bound sialic acid (LBSA) and protein-bound sialic acid (PBSA) were measured in both groups. For evaluation of immune response peripheral blood mononuclear cells (PBMCs) were isolated from blood of animals by Ficoll-Hypaque gradient. Finally, the respiratory burst of phagocytes and the proliferation of lymphocytes were evaluated in PBMC population. Compared to controls, sialic acid concentrations showed significant increase (p< 0.05) in infected buffaloes. Parasitemia rate was positively correlated with sialic acid concentrations. The data of NBT reduction assay and MTT proliferation method showed that the respiratory burst of phagocytes and the proliferation of lymphocytes were significantly decreased in infected buffaloes. The parasitemia rate was also positively correlated with decrease in NBT and MTT assays compared to control group. This study demonstrated that T. annulata infection induced marked and persistent elevations of serum sialic acid concentrations. It seems that increased of serum sialic acid concentrations during parasitemia alter receptor-ligand interactions, which are known to play important role in immune response. Furthermore, sialic acid would indirectly inhibit the action of leukocytes consequently promote the evasion of the immune response and persistence of parasite in the host. It seems that both the innate and acquired immunity in buffaloes infected with T. annulata were diminished in a positive correlation with parasitemia rate.

**Keywords:** buffalo, *Theileria annulata*, parasitemia, sialic acid, acquired immunity, innate immunity

DETECTION OF THEILERIA EQUI AND BABE-SIA CABALLI IN HORSES AND DONKEYS BY MI-CROSCOPIC AND MOLECULAR METHODS IN URMIA SUBURB, WEST AZERBAIJAN PROVINCE, IRAN

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Equine piroplasmosis (EP) is a tick-borne disease caused by hemoparasites Babesia caballi and Theileria equi. The disease affects all members of the Equus Genus (horses, donkeys and zebras). The aim of this study was to determine prevalence of B. caballi and T. equi from horses and working donkeys of Urmia suburb, West Azerbaijan province, Iran. From April to September 2011, a total of 240 and 120 blood samples collected randomly from horses and donkeys, respectively. The specimens were transferred to the laboratory and the blood smears stained with Geimsa. Extracted DNA from each blood sample was used in multiplex PCR in order to confirm the presence of B. caballi and T. equi. Microscopic observation on 240 blood smears of horses determined 15 (6.25%) and 5 (2.8%) samples were infected by T. equi and B. caballi, respectively. The mixed infections occurred in 2(0.83%) samples. The results of the PCR assays of horse blood samples showed 26 (10.83%), 14 (5.83%) and 4 (1.66%) were distinguished as T. equi, B. caballi and mixed infection, respectively. Microscopic observation on 120 blood smears of donkeys showed 2 (1.66%) samples were infected by T. equi. B. caballi infection was not seen in blood smears of donkeys. The results of the PCR assays of donkeys blood samples showed 3 (2.5%), 1 (0.83%) were distinguished as T. equi, B. caballi, respectively. Our findings indicated that T. equi and B. caballi were prevalent among horse and donkeys population.

Keywords: Theileria equi, Babesia caballi, multiplex PCR, horse, donkey, Iran





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# PREVALENCE OF INTESTINAL PARASITES AMONG IMMUNOSUPPRESSED PATIENTS UN-DERGOING CHEMOTHERAPY IN KHUZESTAN PROVINCE, IRAN

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Enteric parasites are important pathogens in patients with human immunodeficiency virus infection and immunosuppressed patients. These organisms can cause mortality and morbidity in these patients. There are few reports on the prevalence of intestinal parasites in cancer patients in Iran. The aim of this study was to determine the prevalence of intestinal parasites in cancer patients undergoing chemotherapy in Khozestan province, Materials & Methodes: In this cross sectional study 200 cancer patients admitted to Golestan hospital in Ahvaz from March 2013 to April 2014 were included. A single stool specimen was collected from subjects. The specimens were examined with direct smear observation using Lugol's solution, normal salin and flotation with saturated sucrose concentration methods. The correlation of the prevalence of the parasites with other categorical factors were analysed with Chi- square test ( $\chi 2$ ). The overall prevalence of intestinal parasites was 16.5%. Blastocystis huminis with 5% was the most prevalent parasites followed by Cryptosporidium parvum, Giardia lamblia, Isospora belli and Strongyloides stercoralis with 3%, 2.5%, 0.5%, and 0.5%, respectively. In 3.5% and 1.5% of subjects co-existence of Giardia with B. huminis and Cryptosporidium were detected, respectively. Of 200 patients, 39 (19.5%) had diarrhea and intestinal parasites were significantly more common among patients with diarrhea (P < 0.05). The results of this study highlight the importance of testing for intestinal parasites among cancer patients, especially those with low immunity presenting with diarrhea needing rapid diagnosis and treatment.

Keywords: cancer, chemotherapy, intestinal parasites, Khuzestan

## COMPARATIVE EFFICACY OF TWO FIXATIVES FOR PERMANENT STAINING OF FASCIOLA SPP. ADULT WORMS

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Fixatives have a major impact on subsequent processing of dehydrating, clearing, and mounting of parasites. They make the parasite more easily stained. This is the most important step for keeping the size in morphometric measurements. The present study aimed to evaluate the effect of 10% formalin and FAAL fixatives on the size of adult *Fasciola* spp. Adult Fasciola isolates were collected from the bile ducts of infected sheep at Yasouj abattoir and fixed with either FAAL or 10% formalin. The length and width of each fluke was measured regularly up to two months. Collected data were analyzed by SPSS, ver. 16, using repeated measure method. Comparison of two fixatives revealed that FAAL with ascending trend and 10% formalin with descending trend changed the size of parasites after 3 weeks of treatment. The differences in the size variations in two fixatives up to three weeks of fixation were statistically significant (P<0.05). After three weeks, constant trend was seen in the size of the flukes.Findings of this study revealed that both 10% formalin and FAAL can be used as fixatives for morphometric analysis of the Fasciola spp. but the time of measurement after fixation is very crucial.

Keywords: FAAL, formalin, fixative, Fasciola





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EVALUATION OF THE IMMUNOREGULATORY EFFECTS OF ES ANTIGENS OF MARSHALLAGIA MARSHALLI IN A MOUSE MODEL OF EXPERI-MENTAL ALLERGIC ASTHMA

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According to hygiene hypothesis, vaccination and proper sanitation will result in decreased chronic parasitic infection but can impair the development of immune regulatory network and predispose the autoimmune and allergic diseases. Materils & Methods: Allergic airway inflammation is induced in Balb/c mice by sensitization with ovalbumin. The effect of the M. marshalli ES Ags on the development of asthma is evaluated by analyzing serum antibody titers, lung histology, cell counts and cytokine levels in the bronchoalveolar lavage fluid. This study shows that airway inflammation in OVA sensitized/challenged mice is decreased after the application of ES antigens M. marshalli. The intense infiltration of inflammatory cells into the lungs was observed near the bronchioles and vessels of mice sensitized and challenged with OVA. In contrast, less inflammatory cell infiltration was observed in the lungs of mice immunized with ES antigens. Moreover, in comparison with control group, ovalbumin specific serum IgE and Th2 cytokines level was decreased but Treg cytokines level was increased. This study could provide new insights into immune modulation by the M. marshalli, with suppressive potentiative airway inflammation in mice during the development of asthma. The identification and characterization of parasite-derived immune-modulating molecules might have potential for designing novel prophylactic/therapeutic strategies for immune-mediated diseases.

Keywords: Marshallagia marshalli, allergic asthma, immunoregulatory DETERMINATION OF KNOWLEDGE LEVELS REGARDING TICKS AND CRIMEAN-CONGO HAEMORRHAGIC FEVER AMONG VETERINARI-ANS, NURSES AND NURSING STUDENTS IN TURKEY

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Health care workers are under high risk of vector-borne diseases. Ticks and tick-borne diseases are important public health problems and Crimean-Congo Hemorrhagic Fever is an emerging, zoonotic disease in Turkey. The aim of this descriptive study was to determine the knowledge levels regarding ticks and Crimean-Congo Hemorrhagic Fever among veterinarians, nurses and nursing students in Turkey. A crosssectional survey was performed with a questionnaire consisting of 20 items about agent, epidemiology, clinical features and prevention methods of CCHF and also basic information about ticks. Data were collected from 53 veterinarians all across Turkey, 62 nurses from Bitlis and Amasya state hospitals and 133 nursing students from Health College of Bitlis Eren University by face-to-face interviews. The people in the groups knew general characteristics of CCHF, transmission of the disease and medical response with high percentage. Nursing students failed at the questions about general characteristics of ticks. Fourth-grade students were found more successful at 13 questions than 1st, 2nd and 3rd grade students, also nurses from Amasya were more successful at 16 questions than nurses from Bitlis. Veterinarians gave correct answers to 12 questions out of 20, nurses 6 out of 20 and students 1 out of 20 with the highest percentage and nurses and veterinarians answered one question with equal success.We have found that the participants have insufficient knowledge about ticks and CCHF. It is recommended that health care workers should be informed about ticks and tickborne diseases after graduation, also nursing students should receive education about related subject.

Keywords: CCHF, tick, veterinary, nurse, student





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IDENTIFICATION OF WATER PROTOZOA IN MILITARY TROOPS LOCATED IN ŞIRNAK PROV-INCE OF TURKEY

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Several parasitic protozoa are transmitted through water and Giardia spp., Entamoeba spp. and Cryptosporidium spp. can cause water-borne diseases in humans. This study aims to determine water protozoa in military troops located in Şırnak province of Turkey. On November and December 2013 water samples collected from 30 military troops into the bottles of 5 liters. Water samples were filtered by the membrane filtration system and the membrane was washed in 20 ml of the same water sample. Then it was centrifuged at 8000 rpm for 10 minutes. The supernatant was discarded and the pellet was examined under the light microscope after stained with via Kinyoun Acit fast and Giemsa. Two protozoan species and one diatome were detected in samples. Cryptosporidium oocysts were detected in 12 of 30 samples and Giardia spp. cysts in 6 of 30 samples. Diatome was detected in one sample and identified as Navicula lanceolata. This study provides the first detection of these species in water samples in the region and we suggest further studies to determine water protozoa in the region and all over the country.

Keywords: water, protozoa, Şırnak, Turkey

PREVALENCE OF HAEMOPROTEUS COLUMBAE IN DOMESTIC PIGEONS IN ZABOL COUNTY IN SISTAN AND BALUCHESTAN PROVINCE IN SOUTHEAST OF IRAN

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Pigeons are cosmopolitan birds and can be found easily in every area except poles. The aim of this study was to determine the prevalence of *Haemoproteus columbae* in pigeons from July to September 2014 in Zabol southeast of Iran.Blood samples were obtained from wing vein and thin smears were prepared from them. All smears were stained by Giemsa and examined by immersion. During the period of this study 150 pigeons (75 female and 75 male) were investigated.This study showed that the prevalence rate of *Haemoproteus columbae* was 45.3% (68/150). Prevalence of *Haemoproteus columbae* was higher in female pigeon (46.6%) than male (44%).Further study needs to be conducted to list different species of this parasite and determine the economic losses due to the parasitic infection along with control strategies.

Keywords: Haemoproteus columbae, pigeons, Baluchestan.





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## PRACTICAL PARASITOLOGY COURSES AND IN-FECTION WITH INTESTINAL PARASITES IN THE STUDENTS

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Students who are in education in research or educational laboratories of parasitology, as well as health care workers providing patients with care, are at the risk of becoming infected with parasites through accidental exposure, which may or may not be recognized when they occur. Parasitic infection can cause significant morbidity and can potentially lead to growth, nutritional, and developmental impairment. The main purpose of this study was to identify possible positive cases of intestinal parasitic infection among students who took practical parasitology courses compared with two control groups in Lorestan University of Medical Sciences, Lorestan province, west of Iran, in 2013. A total of 272 subjects from various majors were invited to participate in the study. Data on age, sex, place of residence, taking or not taking practical parasitology courses, and hand washing after practice was collected via questionnaires. Three stool samples on three consecutive days were collected from each student. To diagnose the presence of parasitic infections, direct fecal smear (DFS) and formalin-ether concentration test (FECT) were used. The prevalence rate of intestinal parasitic infection among the students was 19.11% while this rate among the students who took practical parasitology courses (48/214, 22.42%) was higher than the control group (4/58, 6.9%). The most frequently observed intestinal parasites were Blastocystis hominis (5.51%), Giardia lamblia (4.41%) and Entamoeba coli (3.67%), respectively. No statistically significant difference was observed between age groups, sex and place of residence in the infection with intestinal parasites. According to the results of this study, infection with protozoan parasites among students of different majors was higher than helminth parasitic infections. Moreover, the findings showed that transmission of pathogenic and nonpathogenic parasites in the educational course of practical parasitology could take place and must be taken into careful consideration. Hence, health education and prevention methods about routes of infection with intestinal parasites in every place especially in parasitology laboratories must be considered.

Keywords: practical parasitology courses, intestinal parasites, students, Iran

# PARASITIC CONTAMINATION OF RAW VEGE-TABLES FROM ZANJAN MARKETS, IRAN

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Vegetables have very important place in human's healthy diet but unfortunately, their complex surface and porosity facilitate pathogen attachment and their survival. This study aimed to detect the parasitic contamination in common raw vegetables in Zanjan since soil transmitted parasites are endemic in this region. A total of 352 local vegetables and those harvested elsewhere but distributed in this region were selected for this survey; including: Leek, Parsley, Basil, Mint, Radish, Cress and dill. Samples were collected randomly from markets in different parts of the city. The edible part of each vegetable samples was separated according to the household practice. Samples were immersed in physiological saline solution (95% NaCl) and sedimentation was allowed at room temperature for 24 hours. The top water was discarded and the remaining washing water examined via sedimentation and floatation method then observed by light microscopy. Among 352 samples, 54% were positive for pathogenic and non-pathogenic organism but 2.8% of them were positive for parasitic organism. The parasites were: 3 Trichostrongylus eggs, 2 Hook worm eggs, 2 Eimeria oocysts, 1 Sarcocystis oocyst, 1 Strongyloides larva and 1 trematoda like egg (0.28%). Vegetable contamination with parasitic organisms in this area is less than other parts of Iran, which may be attributed to usage of clean water and not sewage for irrigating in this region. The results showed that the level of contamination varies in different seasons in that the highest number of contaminated samples was observed in autumn (p < 0.05). The reason is that people consume local vegetables in spring and summer but in the cold seasons vegetables from other regions especially Khozestan province are used.

Keywords: parasitological contamination, raw vegetables, Zanjan





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# HABRONEMA MUSCAE LARVAE: LIFE CYCLE IN MUSCA DOMESTICA

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Dipteran insects are vectors of diseases having major importance for animals and humans. Among parasitic nematodes transmitted by muscids, Habronema spp. affect equids all over the world. In the past few years several gaps have been filled in our knowledge on equid H. microstoma and H. muscae, although different aspects are still poorly known, especially their life cycle in the intermediate hosts. The present paper describes the morphological modifications occurring during the larval development of Habronema muscae (Nematoda: Habronematidae) in Musca domestica (Diptera: Muscidae), along with the reactions caused by parasitism and the migration route of the nematodes inside the flies. Houseflies were reared on feces of a H. muscae-infected horse, then dissected and processed by histology. Three different larval stages of H. muscae were recovered, measured and described. The encapsulation of larval nematodes was found in the third larval instar (L3) of M. domestica and Cryptocephalic pupa. The mature capsules were observed in dipteran L3, pupae and mainly adults. In M. domestica adults of 1 day-old or more, an active rupturing of capsules by H. muscae L3 and the migration to the head through the circulatory system and insect hemocoel were observed. Infective H. muscae L3s remained exclusively in the head of M. domestica adult of 5 days-old or more. The host-parasite relationship between M. domestica and H. muscae is ecologically and evolutionarily well established, also the clear reaction of M. domestica to stabilize H. muscae and also the altered longevity of infected dipterous by this parasitism should be considered. These evolutionary features are extended to the relationship between equids and H. muscae, because the L3s of the nematode show a distinct morphological preparation to infest the vertebrate hosts.

Keywords: Habronema muscae, life cycle, Musca domestica

COMPARISON OF THREE STAINING METHODS FOR THE DETECTION OF INTESTINAL MICRO-SPORA SPP.

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Microspridia are obligate intracellular spore-forming protozoa. They have been recognized as human pathogens particularly in immunodificient patients. This study aimed to compare three staining methods including: calcofluor white, chromotrope and quick hot Gram chromotrope used in diagnosis of intestinal microsporidial spores. One hundred and seventy five stool specimens were collected from patients referred to laboratory of intestinal protozoology at the School of Public Health, Tehran University of Medical Sciences during 2012-2013. All of specimens were evaluated by nested PCR. The formalin-fixed stool samples were prepared from each specimen and dried at room temperature for 10 min, followed by 10 min methanol fixation. All the collected stool samples were evaluated blindly by calcofluor white, chromotrope and quick hot Gram chromotrope staining methods separately. Microsporidial spores were recognized using chromotrope, quick hot Gram chromotrope and calcofluor white, in16 of 18 (88.8%), 17 of 18 (94.4%) and 18 of 18(100%) samples that were positive by nested PCR respectively. Regarding 14 stool samples that were negative by nested PCR, 14 cases were negative by chromotrope and quick hot Gram chromotrope and 13 samples were negative by calcofluor white. One discordant sample interpreted as false positive.Calcofluor white staining had the best performance for the detection of intestinal Microsprora spores and can be used as initial screen test for the detection of intestinal Microspora spp.

Keywords: Microspora, staining, chromotrope, Gram stain





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# PREVALENCE OF BLASTOCYSTIS HOMINIS IN-FECTION IN TABRIZ

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Blastocystis hominis is one of the most common intestinal protozoan parasites in human and various animals which has a worldwide distribution and is often isolated from fecal samples in the parasitology laboratory. Given the relatively high prevalence and emphasis on pathogenicity of Blastocystis hominis especially in immunocompromised patients in recent studies, this study was performed to investigate the prevalence of Blastocystis hominis in Tabriz in 2009-2010. The sample size for this this descriptive cross-sectional study was calculated as 558 samples using statistical formula. Stool samples from each of the cases were examined 3 times using direct examination with saline and Lugol's solution, staining with trichrome and formalin-ethyl acetate concentration method. Out of 558 cases, 146 (26.17%) had Blastocystis hominis and 412 (73.83%) were negative. Abdominal pain (49.4%), anorexia (35.8%) and nausea (33%) were the most common symptoms in cases infected with Blastocystis hominis alone.Blastocystis hominis infection was the most common parasitic infection and if ignored can cause many problems for the patients. Therefore, the use of appropriate diagnostic laboratory procedures in hospitals and medical centers can provide effective and accurate diagnosis which will play an important role in the promotion of community health.

Keywords: *Blastocystis hominis*, prevalence immunocompromised patients, Tabriz

# MOLECULAR STUDY OF ZOONOTIC PARASITE OF BLASTOCYSTIS SP. IN BIRDS IN KHORRAMA-BAD, LORESTAN PROVINCE, IRAN

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Blastocystis is a zoonotic protozoan parasite with worldwide distribution that lives in the digestive system. Molecular studies have proved the presence of different subtypes of the parasite. The aim of this study which was conducted for the first time in Iran, was to determine the prevalence of Blastocystis and its subtypes in birds. The study was performed on 748 stool samples collected from birds in Khorramabad, in 2012. Genomic DNA was extracted and examined for the presence of Blastocystis parasite using PCR. In order to determine the Blastocystis subtypes in contaminated sample, seven pairs of primers STS (subtype specific sequence-tagged site) were used. Out of 748 specimens, 1 sample (0.13%) was infected with Blastocystis. Subtype of this Blastocystis was ST2.In this study the prevalence of Blastocystis in birds was very low. In order to clarify the issue, performing similar studies on birds of the province is recommended.

Keywords: birds, Blastocystis, PCR, Lorestan





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#### THE PREVALENCE OF INTESTINAL PARASITES IN HEMODIALYSIS PATIENTS IN BUSHEHR, IRAN

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Hemodialysis patients, due to a dysfunction of the immune response, are prone to a variety of opportunistic infections. Due to paucity of studies on intestinal parasitic infections in these patients, the present study was carried out to determine the prevalence of these infections in patients on hemodialysis in Bushehr. In this cross-sectional study, fecal samples from all hemodialysis patients referred continuously to the dialysis center since Sep 2011 till Sep 2012 were investigated using formalin-ether concentration method. 25 patients (28.4%) were infected with at least one type of intestinal protozoa or worm, 4.5% of them had more than one type of parasitic infection. Statistical analysis showed no relationship between sex and the risk of intestinal parasites. About 17% of infected patients had diarrhea and this relationship was statistically significant.Considering the high prevalence of intestinal parasitic infections among hemodialysis patients in Bushehr and also the high probability of getting infection in these patients, performing periodic examinations and screening measures during dialysis attendance and before kidney transplantation is recommended as a part of routine medical surviellance.

Keywords: prevalence, intestinal parasites, hemodialysis, Bu-shehr

#### PREVALENCE OF INTESTINAL PARASITES AMONG FOOD-HANDLERS IN SHIRAZ, IRAN

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Parasitic intestinal infections are still among socioeconomic problems in the world especially in developing countries like Iran. Food-handlers that directly deal with production and distribution of foods in communities are one of the most important sources for transmission of parasitic infections to humans. The aim of this study was to determine the prevalence of intestinal parasitic infections (IPIs) among foodhandlers in Shiraz. In a cross sectional study 1021 feces samples were randomly collected from food-handlers In Shiraz. Two different methods, routine direct fecal examination (Lugol's iodine solution) and (Formol-Ethyl Acetate Concentration) was applied as a complementary technique to detect parasites. The prevalence of parasitic organisms was 10.4% in the food-handlers. The most species of protozoan parasite were Entamoeba coli (3.5%) and Blastocystis hominis (3.3%) respectively; meanwhile, only one egg of Hymenolepis nana (0.01%) was detected. The majority of participants were males (57%); however, data analysis showed significant statistical difference in females (11.9% (n=53/444)) than males 9%(n=52/577), (p < 0.024). There was no significant statistical difference in the rate of infection based on education and occupation. The current system for monitoring intestinal parasitic infections in food-handlers is not sufficient and should be improved by establishing digital files consisting demographic information and results of parasitological examinations for epidemiological studies and occupational health management need more attention for changes such as: establishing digital file and parasitic report information meanwhile digital files should be archived for a long lasting period of years to analyzing and future epidemiological study and occupational health management. Continuous annual training courses has a great role in decreaseing the prevalence of parasitic infections in food-handlers

Keywords: intestinal parasite, food handler, prevalence, Shiraz





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# PREVALENCE OF INTESTINAL PARASITIC IN-FECTIONS IN PATIENTS REFERRED TO RAZI LABORATORY OF RASHT, IRAN

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Intestinal parasitic infections are among the most common infectious diseases and major health problems worldwide. The purpose of this study was to determine the prevalence of intestinal parasitic infections in patients referred to Razi Laboratory of Rasht and the impact of demographic factors on them. This descriptive cross-sectional study was performed on 370 randomly collected samples (219 from the Rasht and 151 from the sorrounding villages) in 2013. All of the samples were examined by two methods: direct smear and formalin-ether method. Also, demographic characteristics of participants were collected by a questionnaire. Findings of this study revealed that 12.6% of the villagers and 6.4% of the city dwellers had at least one intestinal parasite that shows a significant statistical difference (P<0.01). Giardia lambelia had the most prevalence (%3) and the prevalence of other intestinal parasites were: Strongyloides stercoralis 1.6%, Enterobius vermicularis0.5%, Trichostrogylus spp. 0.3%, Entamoeba coli 2.2%, Iodamoeba butschlii 0.8%, Blastocystis hominis 1.9% and Chilomastix mesnili 0.3%. There was a significant relationship between participants' age, place of residence, and level of education and the prevalence of Strongyloides stercoralis. There was only a significant relationship between participants' age and the prevalence of Giardia and Enterobius vermicularis. These parasites were mainly observed in lower ages. Regarding Blastocystis hominis, a significant relationship between participants' sex and prevalence of this parasite was proved, so that a greater level of infection were seen in male individuals (P < 0.01). According to this study, the prevalence of the infection has been significantly declined over the past years. However, given the relatively high prevalence of intestinal parasites among the villagers, health education, improving the hygiene statue and treatment of infected individuals has great impact on decreasing the infections in rural comunities.

Keywords: intestinal parasites, prevalence, Rasht, Iran.

# PREVALENCE OF INTESTINAL PARASITIC IN-FECTIONS IN REHABILITATION CENTERS IN GOLESTAN PROVINCE

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Study and diagnosis of parasitic infections, especially intestinal parasites, are of particular importance in health planning, control and prevention of the diseases. Given the importance of awareness of parasitic infection in rehabilitation centers in Iran, the present study was conducted to investigate the prevalence of intestinal parasitic infections in disabled rehabilitation centers in Golestan province in northern Iran. A crosssectional study was performed to determine the prevalence of intestinal parasitic infections in four Disabled rehabilitation centers of Golestan province in the first half of 2008. A triple stool samples were collected from each of the 196 subjects out of 566 people resident in disabled centers. Each specimen was examined by direct wet mounting, formalin-ether concentration and trichrome permanent staining and examined by light microscopy. The data were analyzed using SPSS software and chi-square test. No intestinal parasitic infection was found in 172 (87.7%) cases. The overall infection rate of intestinal parasite was 12.3% (24 out of 196 subjects; 7.1% in males and 5.2% in females). Blastocystis hominis 4.2% and Giardia lamblia 3.1% were found most commonly than Chilomastix mesnili (0.5%). In this study prevalence of the pathogenic protozoan and worms were 9.3%. Prevalence of parasitic infections were highest in subjects under 9 years and 20-29 years old (4.1%) and the lowest in 40 years old individuals (0.5 %) (P<0.001). Parasitic infection in mentally handicapped was 9.2% and the physically exercise - mentally 2.5%. Intestinal parasitic infection among the disabled Elderly and people with Down syndrome were not observed. Specific infection rate in mentally handicapped was 75% and physically exercise - mentally 21%. The prevalence of intestinal parasites in people living in disabled rehabilitation centers is not high compared to other social groups in Golestan province. However, due to advers complications of amoebiasis, giardiasis and other parasitic disease, improving personal and social hygiene in local human population is essential.

Keywords: intestinal parasitic infections, rehabilitation centers, *giardia*sis, amoebiasis, Golestan





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PARASITIC CONTAMINATION OF RAW VEGE-TABLES IN SANANDAJ, KURDISTAN PROVINCE IN 2013

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Parasitic diseases are among of the most important health problems all over the world, particularly in developing countries. Since, consuming of raw vegetables is the most common way of the parasitic infections, this study aimed to survey the presence of parasites on raw vegetables of Sanandaj city (west of Iran) in 2013. In this descriptive study the samples were taken randomly from 60 vegetables stores (6 sample from each). Parasites obtained by putting the samples in distilled water and centrifugation of the precipitated materials in 5000 rpm. The samples then were investigated under optical microscope. The data were analyzed using Spss16. The obtained parasites on the vegetables were Free-living nematodes (12.2%), Entamoeba coli cyst (1.7%), Giardia lamblia cyst (0.8%), Blastocystis hominis (0.8%), and eggs of Dicrocoelium dendriticum (0.8%). Totally 16.3% of the vegetables were contaminated with either pathogenic or non-pathogenic parasites. Interestingly, most of the contaminations (13.6%) were found on vegetables imported from other part to the Kurdistan province. Moreover, lettuce and cabbage were the most frequent consumed vegetables that contaminated with parasitic agents. We found that the rate of contamination is much higher in winter and less common in spring. According to the collected data the rate of vegetable contamination in Sanandaj was lower than those reported from other Iranian cities Shahrood, Zabol, Ahvaz and Arak. Improving the public health standards, implementing the public health education programs, proper washing of consumed vegetables and avoiding the use of human and animal manure as fertilizer have crucial role in decreasing the prevalence of parasitic infections in human population.

Keywords: vegetables, parasitic infections, Giardia lamblia, Kurdistan

# BLOOD PARASITES OF RODENTS IN RAZAN PLAIN, WESTERN OF IRAN

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Rodents are represented by many families which relatively have small and medium size. They are acting as reservoir of ecto and endo parasites of medical and veterinary importance. The rodents dwell in human altered habitats and hence it can play an important role in distribution of the human pathogens. The aim of this study was to determine the prevalence of blood parasites of rodents for the first time in Razan plain. We conducted a fieldwork on the parasites of three types of rodents from December 2010 to November 2011. The captured animals were anesthetized and after recording their gender, their blood samples were taken using the heart puncture method. Then, blood smears prepared and stained by the standard Giemsa staining method, examined microscopically and identified using morphometric reference parameters. A total of 68 rodents, belonging to three species, the Wood Mouse, Apodemus sylvaticus (n = 36), the house mouse, Mus musculus (n = 30), and European water vole, Arvicola terrestris (n = 2) were captured. The blood parasites were found in 10 (14.7%) of the specimens. One (1.47%) mouse (A. sylvaticus) was infected with Trypanosoma grosi which was identified for the first times in Iran, and 9 (13.2%) mice (M. musculus) were infected with Bartonella sp and in two A. terrestris examined no haemoparasite was found.Rodents could be as reservoir hosts for vector borne parasites and study on their zoonotic haemoparasites has medical importance.

Keywords: Apodemus sylvaticus, Mus musculus, rodents, Razan plain, Iran





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ACUTE TRYPANOSOMIASIS DUE TO TRYPANO-

#### SOMA THEILERI IN A DAIRY CATTLE IN KUR-DISTAN PROVINCE, IRAN (FIRST REPORT)

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In July 2013 a seven year-old dairy Holstein cattle was referred to the Veterinary Clinic in Baneh Kurdistan Province. The cow had a history of pyrexia, anemia, weakness and lymphadenopathy. Laboratory findings revealed evidence of anemia and a lymphocytosis. Examination of Giemsa stained blood smears revealed the presence of a large number of *Trypanasoma theileri*. Also, the animal was negative for other blood protozoan and anaplasma infections. After administration of Diminazene (5 mg/kg/IM two doses) along with Dexamethazone for 48 hours clinical cure was observed. Our report indicates that *Trypanasoma theileri* should be considered as a pathogen for cattle unlike common believes.

Keywords: trypanosomiasis, Trypanosoma theileri, Kurdistan

# PREVALENCE OF BLASTOCYSTOSIS, AMOEBIA-SIS AND GIARDIASIS AMONG 1-12 YEAR-OLD CHILDREN REFERRED TO BU-ALI HOSPITAL, ARDABIL, IRAN

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Blastocystosis (Blastocystis hominis), amoebiasis (Entamoeba histolytica) and giardiasis (Giardia lamblia) are considered as important parasitic infections in children, because of their pathogenicity and high prevalence worldwide, especially in developing countries. The most important symptoms of blastocystosis in children are acute diarrhea, enteritis, nausea and vomiting. The symptoms of amoebiasis are dysentery and colitis which could lead to death in children. Giardiasis is one of the most prevalent and important intestinal parasitic infection among 3-11 year-old children. The outcomes of giardiasis in children are steatorrhea, hypovitaminosis, loss of weight and growth retardation. In this descriptive and crosssectional study performed in March 2013 to March 2014, 7700 specimens from children referred to Bu-Ali hospital in Ardabil collected and examined by Iodine wet mount and formalin-ether methods. Of 7700 studied children, protozoal infections were present in 113 cases (1.44%), from which 23 cases (0.30%) were infected with Blastocystis hominis, 21 cases (0.27%) with Entamoeba histolytica and 20 cases (0.26%) with Giardia lamblia. 60.8 percent of blastocystosis infected children were boys and 39.2% were girls. 61.2 % of the cases which infected with amoebiasis were male and 38.1% were female. In addition, 80 percent of giardiasis cases belonged to boys and only 20 percent to girls. Our study confirmed the presence of blastocystosis, amoebiasis and giardiasis among 1-12 year-old children in Ardabil. It appears that boys more than girls are at expose of the infections.

Keywords: blastocystosis, amoebiasis, giardiasis, children, Ardabil





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PARASITOLOGY EDUCATION AND LEARNING

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Infographics or informing graphics explain the data in a pictorial language; using this method, persons are able to receive a large quantity of information in a simple way and memorize them actively, because human brain shows more tending to analyze and store as visual data. Most theoretical topics propounded in medical parasitology such as morphology, life cycle, control and prevention, and even pathogenesis, diagnosis and treatment have the capability to be observed by graphs. This study was designed and carried out as an electronic portfolio on application of Infographics in medical parasitology Learning. This study was carried out during the first semester of academic year 2012-2013 with participation of Bsc level of lab sciences students. At first, the principles of Infographics were taught to them as in detail and applicable; then they were asked to prepare and provide toxoplasmosis topic infographically using Photoshop CS5<sup>™</sup> software. At the end of final exam, quantitative and qualitative parameters and also efficacy of this method in learning theoretical subjects were assessed using researcher-made questionnaire. The validity of the questionnaire was obtained by content validity and the reliability was determined by Cronbach's Alpha ( $\alpha$ =0.77). The raw data were analyzed by the SPSS version 19 software. The findings showed that all research units recognized infographics effective in improving attitudes and accelerating motives to study; more than 90% of them have recognized the efficacy of this method high and very high in increasing concentration in the classroom, reducing anxiety during study, remembering the taught concepts and stability in learning. Also 81.8% of research units have announced their satisfaction as high and very high in relation to preparing infographics as an electronic portfolio. 72.7% of the students have considered the contents used in preparing infographics as important points in the text books.Infographic electronic portfolio as an acceptable and satisfactory method for the learners is able to provide platform for learning based on thinking.

Keywords: infographics, electronic portfolio, medical parasitology.

#### EFFECT OF CLIMATE CHANGE ON WETLAND AQUATIC INSECT DENSITY AND POPULATION

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Climate change affects on plants, distribution of the vectorborne diseases, and density and population of animals. Wetlands are known as reservoirs of the insect biodiversity. Thus, they would be suitable centers for assessing the effect of climate change on density and population of aquatic insects. So the present study was designed to assess the effect of climate change on density and population of aquatic insects in Shadegan and Hawr Al Hawizeh or Hawr Al Azim wetlands during October 2011 to September 2012. The insect samplings were conducted from six different sites of the wetlands. Adult and premature insect stages were collected by long-handed wide- and a modified student D-form small mesh net in the lotic, lentic and on the aerial and floating parts of the plants and identified under a dissecting microscope using scientific resources and morphology-based identification keys. Meteorological data including mean daily temperature, mean relative humidity, rainfall were obtained from Shadegan synoptic weather station. Also mean water temperature was calculated by measuring water temperatures of the wetland-selected sites. Totally, 18,534 of the arthropod different life stages including 12 orders containing 51 families were collected. Results showed that the abundance of aquatic insects gradually increased by the reduction of the average daily temperature, increasing the mean relative humidity and precipitation, and reduction the average evaporation from October 2011 to April 2012, and conversely gradually decreased by the increasing of the average daily temperature and reduction of the mean relative humidity and precipitation, and increasing the average evaporation from April to September 2012. Also population of insects turned mainly to families that have low level biological indices and they were symbol of unclean water in the autumn. While in the spring, conversely, they mostly changed to families that have high level biological indices and they were symbol of clean water. The present study showed an optimum condition for growth of the aquatic insects in the spring. When the differences between the average daily and water temperatures were at the minimum in the April the aquatic insect abundance reached to their maximum. As a result, the population and density changes of aquatic insects were due to the effect of climate change during the year.

Keywords: aquatic insect, climate change, density, population, wetland





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### PREVALENCE OF INTESTINAL PARASITIC IN-FECTIONS IN PRIMARY SCHOOL CHILDREN, BUSHEHR, IRAN

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The prevalence of intestinal parasites is high among children because of their weak immune system and close contact with contaminated soil and failure to carry out hygienic principles. This study was conducted to determine the prevalence of intestinal parasites and the effects of some factors among primary school children in Bushehr. After coordination with the education office, schools were randomly selected from different areas and fecal samples were collected from 203 male and female students at different educational levels. Samples were examined by formalin-ether sedimentation technique. Data were collected by questionnaire and analyzed using SPSS 18.0 software and Chi square test. About 25.1% of cases, infected at least with one type of intestinal parasites and 5.9% of them infected with more than one species. The highest prevalence was obtained in children in educational levels of 4 and 5. There was no significant relationship between infection with some clinical symptoms such as abdominal pain, appetite, nausea and parents' education but a significant difference was found in regard to the number of family members. Due to the high prevalence of intestinal parasitic infections among school children and knowing that these parasites cause anemia and perturbation in nutrients absorption, growth and learning capacity, implementation of health education courses for parents and improving the hygienic standards are necessary for reducing the the capacity of transmission.

Keywords: prevalence, intestinal parasites, primary school children, Bushehr, Iran

# THE FIRST REPORT OF HAEMATOZOA IN MI-GRATORY AND NATIVE BIRDS IN ISFAHAN PROVINCE, IRAN

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The avian haematozoa, with worldwide distribution, are able to infect a wide range of birds. There are various types of birds hosting haematozoa in Iran. The current study aimed to find whether the avian haematozoa exist in Isfahan province, central Iran. Since the study was the first of its kind in Isfahan, judgmental sampling and accordingly Semirom, Chadegan, Fereydunshahr, Khoomeynishahr, Isfahan and Feridunshar were selected as study areas. In this study, birds belonging to five poultry families including ducks, turkeys, geese, hen/rooster, and pigeons -considered native domestic birds -and a group of waterfowl migratory birds were studied. Blood samples were collected from 275 birds between May and June, 2013. Then, a blood film was prepared from each bird, stained and examined under light microscope. The infection intensity was also determined for each of the infected birds. Two types of haematozoa, namely Aegyptianella (2.9%) and Plasmodium (3.6%) were found in the blood smear of the infected birds. The results revealed that the infection intensity was very low in all the infected cases. Further, the highest rate of the infected cases was related to the turkeys (12.2%). In the geese, of course, no infection was observed. Feridunshar, with 14.2%, was the city having the highest rate of infected cases. In Semirom, however, no infected cases were observed.Based on the results, more studies are necessary to clarify the status of various haematozoa species in other parts of Iran.

Keywords: haematozoa, birds, Isfahan, Iran





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# HELMINTHES INFECTIONS OF COLUMBA LIVIA IN LAHIJAN, GUILAN, IRAN

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Coumba lvia is a bird with a wide distribution in Iran. Recognizing diseases and pathogenes of this bird is of high importance. The aim of the investigation was to study the parasitic infections of Columba livia. This survey was conducted in Lahijan city, northern area of Iran. Sixty Columba livia in four parts of the city randomly were selected, during the year 2011-2012. Gastrointestinal tract from the esophagus to the rectum was evaluated. Nematodes were transparated in lactophenol and cestodes were identified after staining, by diagnostic keys. The helminth parasites of the gastrointestinal tract were isolated and characterized. They included Cestoda: Raillietina magninumida (%54.5), Raillietina echinobothrida (45.4%) , Raillietina tetragona (%45.4), and Nematoda: Acuaria spiralis (%7.6), Ascaridia columbae (%50), and Capillaria obsignata (%38.4). The study showed high rate of infection in this bird which requires further studies.

Keywords: helminth infection, Columba livia, Guilan

#### HELMINTH INFECTIONS OF HEDGEHOGS, ER-INACEUS EUROPAEUS IN HAMADAN, WEST OF IRAN

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Hedgehogs (Erinaceus europaeus) are from Erinaceidae family and familiar groups of mammals. These mammals usually are widely distributed throughout the world in wild life and some are even kept as pets. This animal can be considered as a reservoir for some wide variety of zoonotic pathogens in medical and veterinary fields. Thus, the objective of the current investigation was to prepare a list of parasitic helminthes of hedgehogs for the first time in Hamadan region, west of Iran. The present survey was conducted on the parasites of eight (four males and four females) road killed hedgehogs in Hamadan region during 2011-2012. All of internal organs were dissected and scrutinized for helminthes. The nematodes collected and then preserved in 70% ethanol and then cleared in lactophenol solution. Then obtained Acanthocephals fixed in AFA solution, stained by acetocarmine and drowned with the aid of drawing tube for identification. A total of eight hedgehogs (Erinaceus europaeus) were examined and 66.7% of them harbored helminthes of digestive tracts. Physaloptera clausa (55.6%) observed from stomach and Nephridiacanthus major (22.3%) were identified in small intestine.Some of helminthes associated with hedgehogs is zoonotic and have importance for public health.

Keywords: helminth infections, Erinaceus europaeus, Hamadan





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PARASITIC CONTAMINATION OF RAW VEGE-TABLES IN ASADABAD, WEST OF IRAN, 2014

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Consumption of fruit and fresh vegetables are essential components of a healthy diet. Thus, heath of consumed vegetables can play important role in society public health. The aim of this study was to determine the prevalence of parasitic contamination of farm vegetables to estimate human risk of parasitic infection diseases. In a descriptive cross-sectional study, 383 samples of different vegetables were obtained from 12 vegetable farms around Asadabad city including 10 various types of seasonal vegetables: coriander, radish, spring onion, leek, parsley, tarragon, savory, basil, mint and cress. The collected samples were washed with tap water, immersed in water supplemented with detergent solution (1% sodium dodecyl sulfate, 0.1% Tween 80) and then subjected to parasitic analysis by using sedimentation and floatation methods. SPSS software (version 16) was utilized for data analysis. Parasitic contaminations were detected among 32 (8.4%) fresh vegetable samples which included 16 cases of protozoan cyst (4.2%), 7 cases of worm egg (1.8%) and 9 cases of free living larvae (2.3%). The highest infestation was associated with Entamoeba coli (10, 2.6%) and the lowest were related to Taenia spp. (1, 0.3%) and Dicrocoelium dendriticum (1, 0.3%). Correlation between parasitic contamination of vegetables and type of water consumed in farm vegetables was significant (P= 0.05). The results implicate that consumption of raw vegetables can be considered as a risk factor for parasitic infections. Inadequate hygiene practices, poor sanitation and use of untreated water can widely spread infectious diseases in a community. Use of composted animal manure as fertilizer and irrigation of vegetable farms with safe water can extensively reduce public health concerns. Consequently, public health education, promotion of food hygiene and awareness of hazard of unwashed fruits and vegetables must be considered as an essential in the poor sanitation areas.

Keywords: parasitic contamination, prevalence, vegetables, Asadabad

## PREVALENCE AND INTENSITY OF HAEMATO-ZOAN INFECTIONS IN DOMESTIC PIGEONS IN KHORRAMABAD, SOUTHWEST OF IRAN

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Avian haematozoan parasites including Haemoproteus, Leucocytozoon and Plasmodium are intracellular parasites that are transmitted by biting dipteran flies. These blood parasites are most common in tropical regions and can cause declined productivity in domestic and wild birds. The present study was designed to investigate the prevalence and intensity of haematozoan parasites in domestic pigeons in Khorramabad, southwest of Iran. A total of 93 pigeons were examined for presence of infection. Blood samples were obtained from wing vein and thin smears were prepared from them. The blood smears were air-dried, fixed in methanol and stained with Giemsa. The stained slides were examined for detecting the intracellular gametocytes of blood parasites using optical microscope at x1000 magnification. Two species of avian haematozoan parasites, Haemoproteus and Leucocytozoon, were identified. Sixty six pigeons (71%) were infected with Haemoproteus and 7 pigeons (7.5%) were positives for Leucocytozoon. One pigeon were positive for both Haemoproteus and Leucocytozoon. Among infected pigeons the intensity of Haemoproteus was low in 36%, moderate in 17% and high in 47%. The intensity of Leucocytozoon infection was low in 5 and moderate in 2 cases.Findings indicate that the haematozoan infections in domestic pigeons in Khorramabad are more prevalent than other regions of country.

Keywords: avian haematozoa, Haemoproteus, Leucocytozoon, pigeon, Iran





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## DETERMINATION OF THEILERIA ANNULATA IN NATIVE CATTLE, BY SEMI-NESTED PCR IN ALESHTAR AREA, LORESTAN, IRAN

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Theileriosis is one of the most serious diseases of tropical and subtropical regions. In Iran, this disease causes some economic losses in animal industry anually. Nowadays molecular assays, due to their high sensitivity and specificity rates, are more employed than the traditional methods, Giemsa staining and serology, for detection of piroplasmosis in domestic animals. Therefore, we examined the blood samples of 150 cattle in spring and summer 2013 in order to identify Theileria annulata in native cattle by PCR the region. DNA was extracted from all blood samples. Tbs-s and Tbs-a primers were used to amplify both genus of Theileria and Babesia spacies in PCR. Theileria annulata was confirmed examined by semi-Nested PCR method, using specific primerss.The results revealed 31cases (66.20%) positive. The prevalence of Theileria infection reached the highest level in summer with (7.16%), the highest prevalence of theileriosis was observed in age group (1-3years old), (33.9%) and female cattle (18%) and hybrid races (12%).

Keywords: Theilerios, T. annulata, hybrid cattle, PCR

# THE IMPACT OF BCG VACCINE AND FERTILE HYDATID CYST FLUID IN CLASSICAL AND AL-TERNATIVE MACROPHAGE POLARIZATION

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Classical macrophages (M1) and alternative macrophages (M2) are responsible for various functions in order to maintain homeostasis. BCG vaccine and hydatid cyst fluid can be mentioned as stimulants inducing M1and M2 macrophage polarization. Evaluating the expression of markers such as IFNy and TNFa for M1 phenotype and TGF<sup>β</sup> and IL4 for M2, is one of the confirmatory ways of polarization. The purpose of this study was to verify the polarization of macrophages induced by BCG vaccine and hydatid cyst fluid by studying the gene expression of mentioned markers. Peripheral blood mononuclear was separated by concentration gradiant. Monocytes population was separated by adhesion technique from floating cells in complete cell culture medium. Macrophages were treated by BCG vaccine and hydatid cyst fluid for 8 hours. RT-PCR was performed for confirming the polarization and the expression of mentioned genes of amplified sequences were compared with control groups. Amplification of specific genes in each group confirmed the polarization and also lack of PCR product in negative control samples indicates the absence or a very low expression of this gene in control group. The ability of these stimulants in polarization of macrophages can be used in experimental studies of macrophage polarization. It is also possible to take advantage of this model in order to achieve the goals of therapy and cell therapy in inflammatory diseases and the spread of cancer immunotherapy.

**Keywords:** classical macrophages, alternative macrophages. BCG, hydatid cyst fluid. RT-PCR





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# MOLECULAR DETECTION AND IDENTIFICA-TION OF FOOD-BORNE PARASITES

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Numerous parasites can be transmitted by food which are called food-borne parasites including some protozoa and helminthes. These diseases can affect human public health worldwide. The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) identify a top 10 list of food-borne parasites with the greatest global impact including Echinococcus granulosus, Echinococcus multilocularis, Ascaris lumbricoides, Giardia duodenalis, Trypanosoma cruzi, Opistorchiidae, Toxoplasma gondii, Trichinella spiralis, Taenia saginata and/or solium, Entamoeba histolytica, Cryptosporidium parvum. Some other foodborne parasites are Cyclospora cayetanensis, Anisakis spp. and Diphyllobothrium spp. Because of their small number in the sample, their detection and identification is not always so easy. Also, most parasites are obligatory intracellular and in contrast with bacteria, parasites do not multiply in environment. The current study aimed to recognize parasites which can be transmitted by food and demonstrates ways to prevent their spread. Any isolation and detection procedures are crucial because an enrichment process for parasites is not available. Molecular assays overcome these difficulties and specific limitations. These techniques have been developed for rapid, sensitive and specific identification. The most common molecular methods are polymerase chain reaction (PCR)-based techniques. It is concluded that detailed recognition of food transmitted parasites is a crucial concept due to their pathogenesis properties and effects on human health. Their hazardous characteristics oblige researchers to find out confident solutions for prevention and treatment methods.

Keywords: foodborne parasites, PCR, molecular detection

FREQUENCY OF INTESTINAL PARASITES AMONG PATIENTS WITH GASTROINTESTINAL DISORDERS REFERRED TO THE MEDICAL CENTERS OF NAHAVAND CITY, WEST OF IRAN

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Intestinal parasitic infections have a worldwidedistribution. Poverty, low literacy rate, poor hygiene, lack of access to clean water and warm and humid tropical weather are the causes of the high prevalence of intestinal parasitic infections in developing countries. This study was conducted with the aim of obtaining frequency of intestinal parasites in patient with gastrointestinal disorders in Nahavand city of Hamadan province, west of Iran.A total of 1301 stool samples were collected from patients with gastrointestinal disorders referred to the medical centers during spring and summer 2014. The stool specimens were examined macroscopically and microscopically by using direct smear with normal saline and Lugol's solution, formalin-ether concentration method, Trichrome staining and a modified Ziehl-Neelsen staining technique. The results were analyzed using SPSS version 16 and Chi-square test. Out of 1301 studied individuals, 419 (32.2%) were infected with single or multiple intestinal parasites. The frequency of the observed intestinal parasites was: Blastocystis sp. 350 (26.9%), Entamoeba coli 38 (2.92%), Giardia lamblia 30 (2.3 %), Cryptosporidiumspp. 17 (1.3%), Endolimax nana 17 (1.3%), Entamoeba hartmanni 9 (0.69%), Iodamoeba butschlii 9 (0.69 %), Trichomonas hominis 5 (0.384%), Entamoeba histolitica/E. dispar 4 (0.3%), Chilomastix mesnili 3 (0.23 %), Enterobius vermicularis3 (0.23%), Hymenolepis nana 1 (0.07%) and proglotid of Taenia saginata 1 (0.07%). Blastocystis spp. was the predominant intestinal parasite in the patients with gastrointestinal disorders in this region. Giardia lamblia and Cryptosporidium spp had the highest prevalence among the pathogenic protozoan. The results indicated that intestinal helminthic infection is significantly declined, while protozoan parasites are still prevalent in Nahavand city.

Keywords: intestinal parasites, gastrointestinal disorders, frequency, Nahavand.





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A REPORT ON THE CONTAMINATION OF COMMON CARP (CYPRINUS CARPIO) WITH LIGULA IN ZABOL

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One of the native fish of Hamoon pond and Chah-Nimeh in Zabol, Iran is the common Cyprinus carpio that has been caught for years. This kind of fish is mostly caught in spring and summer although the amount of caught Cyprinus carpio in warm seasons is lower than that in cold seasons. One of the most important cestodes of the fish in fresh waters is Ligula. Crustaceans act as the first and fish as the second intermediate hosts while aquatic birds are the definitive host. The fish are contaminated when eating copepods contaminated with coracidium. This study was performed on 70 Cyprinus carpio caught from Hamoon pond and Chah-Nimeh in 2014 to examine them in terms of the presence of Ligula. The cestodes removed from the fish were put in 70% alcohol and then stained. Of the 70 Cyprinus carpio, 17 fish had long cestodes that were stained with related stain. Using a microscope and diagnostic keys, the Ligula parasites were detected. The maximum and minimum number of cestodes removed from the fish was 7 and 2 cestodes, respectively.Ligulosis is caused by plerocercoids of Ligula intestinalis and has been reported in the fish living in fresh waters in most parts of the world. The plerocercoids with their large size displace the internal organs, disturb the growth of reproductive organs of the fish, and castrate the fish. Considering the pisciculture of Cyprinus carpio in ponds in Zabol and the probable contamination with Ligula, the contaminated fish should be removed from the ponds and prevent the aquatic birds from entering the ponds.

Keywords: Ligula, Cyprinus carpio, plerocercoid, internal organs, aquatic birds, Zabol A REPORT ON THE CONTAMINATION OF SCHIZOCYPRIS ALTIDORSALIS WITH LIGULA IN ZABOL

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One of the native fish of Hamoon pond and Chah-Nimeh in Zabol, Iran is the Schizocypris altidorsalis. This kind of fish is mostly caught in spring and summer although the amount of caught Schizocypris altidorsalis in warm seasons is lower than that in cold seasons. One of the most important cestodes of the fish in fresh waters is Ligula. It evolves indirectly with the first intermediate host of crustaceans, the second intermediate host of fish, and the last host of aquatic birds. The fish are contaminated with this parasite when eating copepods contaminated with corasidium. This study was performed on 125 Schizocypris altidorsalis caught from Hamoon pond and Chah-Nimeh in 2014 to examine them in terms of the presence of Ligula. The cestodes removed from the fish were put in 70% alcohol and then stained. Of the 125 Schizocypris altidorsalis, 11 fish had long cestodes that were stained with necessary stains. Using a microscope and identification keys, the Ligula parasites were detected. The maximum and minimum number of cestodes removed from the fish was 8 and 3 cestodes, respectively. Ligulosis is caused by plerocercoids of Ligula intestinalis and has been reported in the fish living in fresh waters in most parts of the world. The plerocercoids with their large size displace the internal organs, disturb the growth of reproductive organs of the fish, and castrate the fish. Considering the pisciculture of Schizocypris altidorsalis in ponds in Zabol and the probable contamination of these fish with Ligula, the fish contaminated with cestodes should be removed from the ponds and prevent the aquatic birds from entering the ponds.

Keywords: Ligula, Schizocypris altidorsalis, ligulosis, aquatic birds, Zabol





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# STUDY OF BLASTOCYSTIS SPP. IN PATIENTS WITH GASTROINTESTINAL DISORDERS RE-FERRED TO THE MEDICAL CENTERS OF NA-HAVAND CITY, WEST OF IRAN

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Blastocystis is a ubiquitous enteric parasite, with the global distribution that infects humans and animals in developing countries as well as tropical and subtropical regions. It is transmitted mainly through fecal-oral route. Blastocystis infections are more common in persons with poor personal hygiene, thoses exposure to animals, crowded conditions and consumption of contaminated food or water. The most common symptoms in victims are diarrhea, abdominal pain and vomiting. The aim of the present study was to determine the prevalence of Blastocystis spp. among patient with gastrointestinal disorders referred to the medical centers in Nahavand city, Hamadan province, west of Iran. A total of 1301 stool specimens were collected from patients suffering from gastrointestinal disorders, during spring/summer 2014. The stool specimens were examined macroscopically and microscopically by using direct slide smear with saline normal and lugol's solution, formalin-ether concentration and trichrome staining. The results were analyzed using SPSS version 16. Out of 1301 studied individual, 350 (26.9%) were infected with Blastocystis spp. Of these, 684 (52.6%) were male and 617 (47.4%) were female. The prevalence of Blastocystis spp. was 26.5% and 27.2% in male and female patients respectively. Inaddition the frequency of Blastocystis spp. was 26.2% and 25.8% in urban and rural regions respectively. Blastocystis spp. is prevalent among individuals with gastrointestinal disorders in Nahavand city. The prevalence was significantly higher among patients aged ≥12 years old compared to those aged < 12 years (p < 0. 001). No significant associations was found between Blastocystis infection and sex and place of residence. The most common symptoms among the patients with Blastocystis infection were abdominal pain and diarrhea.

Keywords: *Blastocystis* spp, gastrointestinal disorders, prevalence, Nahavand

## MACROSCOPIC EXAMINATION OF SARCOCYSTS IN SLAUGHTERED SHEEP AND GOATS IN TOR-BAT HEYDARIEH IN 2012

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Sarcosystosis is one of the most common zoonotic protozoan disease caused by Sarcocystis species. Because of importance of the parasite in human health, this study was carried out to determine the prevalence of macroscopic sarcocycts in slaughtered goats and sheep in Torbat Heydariyeh slaughterhouse. This cross-sectional study was undertaken on 7619 samples, included 7049 sheep, and 570 goats using macroscopic examination for Sarcosystis. Collected data were analyzed by descriptive statistics. The prevalence of Sarcocystis in sheep and goats was 8.78 %. The ratio of infection was 9.41 % in sheep and 0.7% in goats .The prevalence of the parasite in male sheep and goats were 0%, 0.35% and in female sheep and goats were 9.41 %, 0.35%, respectively. The ratio of infection in young (Under 2 years) sheep and goats were 0.49%, 0.35% and in old (Over 2 years) sheep and goats were 8.92%, 0.35, respectively. Economic losses due to death of animals, sequestration of infected organs and reduction in livestock production is and indication for the existence of underlying health risk factors in this area. So, comprehensive public health measure is needed for controlling the situation in study area.

Keywords: zoonotic disease, prevalence, Sarcosystis





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PARASITIC INFECTIONS OF METACESTODE ORIGIN (HYDATID CYSTS, CYSTICERCUS OVIS, CYSTICERCUS TENUICOLLIS) IN SHEEP AND GOATS SLAUGHTERED IN SLAUGHERHOUSE OF TORBAT HEYDARIEH (1391)

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Parasitic diseases in livestock consist of a wide range of diseases. The most important of them are hydatid cyst, Cysticercus ovis, cysticercus tenucolis. The aim of this study was to determine the prevalence of the parasites infection in slaughtered goats and sheep in Torbat Heydariyeh slaughterhouse and determine the epidemiological status of the parasite in the area and their importance in human health. This crosssectional study was conducted on 7049 sheep and 570 goats. The slaughtered animals were examined by direct observation method. Data analysis was performed using descriptive statistics. According to the results, the total parastic infection in sheep, goats were as followings; hydatid cyst 15.17% and 9.64%, cysticercus tenucolis 6.66% and 44.56% and cysticercus ovis 1.16% and 1.40%. The infection rate in female sheep and goats was (14.31%, 5.26%) for hydatid cyst, (2.48%, 10.17%) for cysticercus tenucolis and (0%, 0.52%) for Cysticercus ovis. In the male sheep and goats hydatid cysts was 86%, 4.38%, cysticercus tenucolis 4.17%, 34.38 % and cysticercus ovis 0.45%, 0.87%, respectively. The prevalence of infection in old sheep and goats (Over 2 years) were 13.77% and 7.5 % for hydatid cysts, 0.66%, 2.63% for cysticercus tenucolis and 0.24%, 0% for cysticercus ovis. In young sheep and goats (Under 2 years) hytadid cysts rate was 1.14 % and 2.1 %, cysticercus tenucolis 6%, 41.92% and cysticercus ovis: 0.92%, 1.4% respectively. This study shows that the prevalence of these parasites is the study region. They cause significant economical losses and can cause significant health problems in the people who live in the area.

Keywords: zoonotic disease, cysticercus tenucolis, cysticercus ovis, hydatid cyst EVALUATION OF INTESTINAL PARASITIC IN-FECTIONS IN OPERATORS PROVIDING SER-VICES, DISTRIBUTION AND SALE OF FOOD, KERMAN, 1392-93

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Health care of food selling operators in food distribution centers plays an important role in the health of society. Given the importance of this as a first step towards understanding and control of parasitic infections as well as due to the lack of accurate information on the amount of parasitic infections in Kerman, this study was conducted on health care applicants. This study was performed in a one-year period (2013-2014). Data related to the examination of parasites of 13700 individuals of centers of preparation, distribution and sale of food and public places in Kerman, were collected from health centers. Out of 13700 people seeking health certificate card a total of 47 (0.34%) were positive, 38 (0.27) males and 9 (0.07%) females. Giardia infection was observed in 43 cases (0.31%), the most common parasite, Entamoeba histolytica / dispar in 2 (0.015%), Hymenolepis nana in 2 (0.015%) and Ascaris in 1 (0.008%). Despite the low incidence of intestinal parasites in the study population, control of parasitic diseases should be regarded as one of the most important measures in health centers. Sanitary regulations imposed by health professionals and health education can reduce the prevalence of parasitic infections. Regular monitoring of food producers and food selling operators for the parasitic infections has a critical role in reducing the risk of transmission in human communities.

Keywords: health care, intestinal parasitic infections, Kerman





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### PREVALENCE OF LIVER FLUKE INFECTIONS IN SHEEP AND GOATS SLAUGHTERED IN TORBAT HEYDARIEH IN 1391

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Parasitic diseases in livestock constitute a wide range of diseases. Among them, dicrocoeliasis and fasciolosis have medical and economic importance. This cross sectional study was conducted to determine the prevalence of hepatic trematode infections in slaughtered ruminants in Torbat Heydariyeh slaughterhouse in 2012. Total samples were 7049 sheep and 570 goats. Prevalence of fasciolosis and dicrocoeliasis were 0.99% and 1.27% in sheep and 2.6%, 5.43% in goats, respectively. Prevalence of infections in female sheep were 0.9%, 0.55%, and in female goats, were 1.05 %, 2.1%. The ratio of dicrocoeliasis and fasciolosis in male sheep were 0.08%, 0.72% and in male goats 1.57 %, 3.3 %, respectively. Prevalence of infection in old (over 2 years) sheep and goats were as followings: (Fasciola: 0.85% 1.75%) and (Dicrocoelium: 0.46%) 1.92%) and in young (under 2 years) goats and sheep were (Fasciola: 0.41%, 0.78%) and (dicrocoeliasis: 0.8%, 3.5%). According to the results, the prevalence of these trematodes were relatively low. Because of the importance of animal husbandry in the region, appropriate parasites control programs seem to be necessary despite the low rate of infection.

Keywords: Fasciola, Dicrocoelium, trematode, fluke, Torbat Heydarieh

# DETERMINATION OF IVERSITY, ABUNDANCE AND DISTRIBUTION OF CERCARIAL INFEC-TION IN LYMNAEA AURICULARIA SNAILS IN WEST AZERBAIJAN PROVINCE, IRAN

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Some freshwater snails considered as the first and often also as the second intermediate hosts of parasitic flukes larvae are of both medical and veterinary importance. Meanwhile Lymnaea auricularia, being one of the most pond snails, also serves as a known intermediate host for many digenean species. So far, a few studies have been carried out on the abundance and diversity of cercarial infection in Lymnaeid snails, in particular L. auricularia, in Iran. In West Azarbaijan province, no study has been undertaken on the abundance of cercarial infection in L. auricularia as yet. Thus, the objective of this study was to elucidate the diversity and regional distribution of larval digenean trematode infection in L. auricularia from different parts of the province. Lymnaeid snails collected from 28 different freshwater habitats located at mountainous and low land areas of north, center and south parts of West Azerbaijan province were examined in order to investigate cercarial infection from May to November 2010. Of 6759 collected Lymnaeid snails, 370 (5.47%) L. auricularia snails were identified. Cercarial infection was found in 276 (74.56%) snails. The results showed that removed cercariae from L. auricularia belonged to Echinostomcercariae (96.38%) and Furcocercariae (3.62%) which were found in two out of 28 sites during the course of study. In summer and fall, the highest prevalence of cercarial infection was recorded for both identified cercariae. It is concluded that L. auricularia could be an important intermediate host of large group digenean trematodes in the region, which is necessary to be considered in the control program of trematode infection.

Keywords: Cercarial infection, Lymnaea auricularia, West Azerbaijan





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GEOGRAPHICAL DISTRIBUTION AND DIVERSI-TY OF LYMNAEIDAE SNAILS IN WEST AZERBAI-JAN PROVINCE, IRAN

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Many species of the freshwater pulmonate snail family Lymnaeidae act as intermediate hosts in the transmission of digenean trematode larvae, including those of the causative agent of fasciolosis. Based on this, current investigation was aimed to determine diversity and geographical distribution of Lymnaeidae snails and role of biochemical indices in habitat of them in West Azerbaijan province, Iran. Simple sampling method was undertaken in north, center and south parts of the province in plain (35 sites) and mountainous (10 sites) regions of spring, summer and fall. A total number of 6759 dextral Lymnaea spp. were collected and identified. The highest percentage belonged to the L. gedrosiana 5029(74.4%), followed by L. stagnalis 889(13.15%), L. truncatula 453(6.7%), L. auricularia 370(5.47%) and L. palustris 18(0.27%) snails, respectively. Geographical distribution of Lymnaeidae snails were recorded in 22 sites of plain and 6 sites of mountainous regions. Biochemical findings indicated that Lymnaeidae snails survived between 15-34 °C in both regions. From acidic to relatively alkalis pH, existence of L. auricularia, L. truncatula and L. palustris were recorded. While L. gedrosiana and L. stagnalis were found in alkalis conditions. Electronic conductivity and salinity of different habitats for Lymnaeidae snails were measured as 284-368 µS/cm and 0.136-1.885 g/L, respectively. It is concluded that further studies are necessary to determine frequency of Lymnaeidae snails' infection due to Fasciola spp. and role of them as intermediate hosts of digenean trematodes in North West of Iran.

Keywords: snail, Lymnaeidae, Fasciola, West Azerbaijan

# THE PREVALENCE OF PARASITIC CONTAMI-NATION OF VEGETABLES CONSUMED IN MALAYER CITY, WEST OF IRAN, 2014

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Consumption of raw vegetables has a potential health hazard. No previous surveys have been conducted to evaluate the prevalence of parasitic contamination of vegetables and its epidemiological aspects in Malayer city, west of Iran. Therefore, the aim of this study was to determine the situation and type of contamination in farm vegetables in this area. This survey was a cross-sectional study with 392 samples of different vegetables including leek, parsley, coriander, radish, spring onion, tarragon, basil, mint, cress and savory. The sample vegetables were collected from 39 vegetable farms in around Malayer city and subjected to parasitic contamination analysis. The samples were washed with tap water and then immersed in water supplemented with detergent solution (1% sodium dodecyl sulfate, 0.1% Tween 80), and tested microscopically after performance of sedimentation and floatation methods. Then, data analysis was done using SPSS software, version 16. The results showed that 14.3% of the vegetable samples were infested with various pathogenic and non pathogenic parasites including protozoan cyst (3.6%), helminth ova (3.8%) and free-living larvae (6.9%). The highest infestation was associated with free living larvae (6.9%) and the lowest were related to Fasciola spp. (0.3%) and Trichostrongylus spp. (0.3%). Cress was the most contaminated vegetable (23%) while no contamination was found in tarragon. Among findings, there were pathogenic and non pathogenic contaminant parasites in samples equal to 5.1% and 9.2%, respectively. The present study showed correlation between parasitic contamination of vegetables and type of water and also fertilizer consumed in farms (P

Keywords: parasitic contamination, prevalence, vegetables, Malayer





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## SEROPREVALENCE OF NEOSPORA CANINUM INFECTION IN DAIRY CATTLE IN LORESTAN PROVINCE, IRAN

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The Neospora caninum parasite causes abortion in cattle in virtually all parts of the world with enormous economic consequences. The purpose of this study was to determine the seroprevalence of antibodies of Neospora caninum in dairy cattle in Lorestan Province, west of Iran. A total of 347 dairy cows were randomly selected. The serum of each case was analyzed for the possibility of the presence of antibody against N. caninum antigen, using the commercial kit: ELISA. The results of the ELISA test indicated that from 347 dairy cattle examined, the antibodies to N. caninum were found in 34 (9.8%). The percentage of seropositive aborted cattle was 13.33%. This study also indicated that there was no significant relationship between seropositivity and such factors as the age, breed, and abortion history of the cattle (P>0.05). Moreover, no significant relationship between seroprevalence of infection among rural and industrial cattle was found (P>0.05). The neosporosis could be one of the possible causes of abortion in cattle. Further studies are recommended to determine the relationship between this parasite and the occurrence of abortion in cattle in the province of Lorestan.

Keywords: Neospora caninum, ELISA, cattle, Lorestan

# PREVALENCE OF BLASTOCYSTIS HOMINIS IN HIV POSITIVE PATIENTS IN KHUZESTAN PROV-INCE AND ITS RELATION TO CD4 NUMBER, 2014

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Blastocystis hominis is a zoonotic protozoan, which is reported in patients with impaired immune system, and clinical symptoms of infection with Blastocystis hominis are more severe than in healthy people. But in this case there is no convincing evidence. Given that there is no recorded information in the province regarding patients with AIDS and prevalence of Blastocystis hominis, this study was designed with a focus on HIV positive people. A descriptive epidemiological research was conducted during November 2013 to May 2014. 268 HIV positive stool samples in Khuzestan province were taken from medical centers in prisons. All of these patients have confirmed HIV and have medical file in medical center. Thus, the number of their CD4 is specified. Samples were concentrated using Formalin-ether technique and prevalence of Blastocystis hominis was specified using microscopic method. Of 268 HIV positive stool samples, 33 (12.3%) were diagnosed with positive Blastocystis hominis using Formalin-ether technique. Average CD4+ cell numbers of patients with Blastocystis hominis was specified as 232 cells per mm3 to mL.Findings in the current study indicate high parasite prevalence in patients with HIV+ and susceptibility of patients with AIDS in confrontation with this parasite. Thus, in coping with infection sources especially for HIV+ patients, safety and healthcare points should be well observed. Also a close relationship was found between Blastocystis hominis and CD4+ reduction especially in HIV+ patients.

**Keywords:** Blastocystis hominis, HIV+ patients, CD4, Khuzestan





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EPIDEMIOLOGICAL STATUS OF THEILERIOSIS IN SMALL RUMINANTS IN KURDISTAN PROV-INCE, WESTERN IRAN: OVER A FIVE YEAR PE-RIOD

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Due to the lack of any information about the prevalence of ovine theileriosis in Kurdistan Province, west of Iran, the present study was aimed to determine the prevalence of the Theileria infection and identification of their species in small livestock in this area. In this survey, 9180 small ruminants, including sheep and goat, were randomly sampled and examined from 600 flocks in Kurdistan Province from June 2008 to August 2013. Thin and thick peripheral blood smears were taken and then stained by Giemsa staining method. A total of 9180 samples were collected, 5460 from sheep and 3720 from goat. Theileria spp. was detected in 1115 (20.4%) of sheep samples and 1393 (37.4%) of goat samples by direct examination of blood smear. Overall, the prevalence rate of Theileria infection was 27.3% (n=2508) in both small ruminants. Moreover, Th. lestoquardi was the most common (65.6%) species and next Th.Ovis (33.6%) found in these animals- using microscopic examination on 100 randomly selected positive smears by semi-nested PCR. The high prevalence of Theileria infection in small ruminants indicates the enzootic status of malignant theileriosis in the western region of Iran.

Keywords: Ovine theileriosis, T. Lestoquardi, T. Ovis, Prevalence, Semi-nested PCR

# PARASITIC HELMINTH INFECTIONS IN NATIVE SHEEP (MEHRABAN) IN HAMEDAN

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Sheep play an important role in national economy in rural regions in Iran. Mehraban is a native breed of sheep in Hamedan province. The aim of this study was to investigate the fauna and frequency of parasitic helminth infections in native sheep (Mehraban breed) in Hamedan, western Iran. From April 2010 to March 2011, the gastrointestinal and respiratory tract of 100 native Mehraban breed sheep was examined using parasitology methods. The overall infection rate was reported 69%. No infection was found in esophagus and rumens. Parabronema skerjabini (22%) and Ostertagia circumcincta (1%) were the maximum and minimum nematode, respectively. In addition, the most dominant of trematode and cestode were detected Fasciola hepatica (13%) and Monezia expansa (13%), respectively. The highest infection rate was reported in summer (84%). There was no significant differences between infection rate and gender (P>0.05). This is the first report of parasitic helminth infections in Mehraban sheep in Hamedan. The present results provide baseline information for the future studies. Also, it seems the scientific strategic campaigning against parasitic infections is an inevitable task.

Keywords: Helminth, Sheep, Hamedan





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# PREVALENCE OF INTESTINAL PARASITIC IN-FECTIONS AMONG HEALTH CARD APPLICANTS REFFERED TO AHVAZ EASTERN HEALTH CENTER DURING 2012-2013

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Intestinal parasitic infections are the most common infections around the world. The conducted epidemic studies in different countries have shown that people's socio-economic status is the cause of intestinal infections occurence. Regarding the importance of epidemiologic studies, this study has been implemented as the first step to identify and control the parasitic infections of health card applicants in Ahvaz city. Material & Method: In a retrospective study recorded information of health card applicants reffered to Ahvaz Eastern Health Center has been gathered and during 2012-2013. All of the parasitic cases (protozoa and worm) have been individually and statistically analyzed according to the different years and seasons of investigation. Among 14614 health card applicants in year 2012, 1693 cases of parasitic infections included 1445 (85.35%) Giardia sp, 31 (1.83%) Entamoeba histolitica/dispar, 29 (1.71%) Oxyuris (pin worm), 60 (3.54%) Hymenolepis nana, and 128 (7.57%) Entamoeba coli. Among the 12444 health card applicants during 2013, 632 cases of parasitic infections including 510 (80.69%) Giardia sp, 20 (3.17%) Entamoeba histolitica/dispar, 19 (3.0%) Oxyuris, 46 (7.28%) Hymenolepis nana, and 337 (5.86%) Entamoeba coli were reported. In both years, the most occurence has been reported in the spring and autumn, and the least during winter. As it is mentioned in the results, the protozoa occurence like Giardia is more than multi-protozoa whose onset may be the result of parasite easy transferring by water and nutrients and the parasite cyst resistance under the bad conditions as well as its simple cell division. However, one reason for the low level of pinworm report is the lack of correct diagnosis, i.e. scotch tape. The outbreak has been in great rate during the summer and autumn, which may be the result of environment appropriate temperature for the cyst, or parasite, while during the winter the parasite outbreak has reported less because of cold weather and dryness. The occurence percentage in 2012 is more than 2013, which may be the result of improving the individual healthcare level and the warmth of earth.

**Keywords:** Intestinal parasitic infections, prevalence, health card applicants, Ahvaz

# **USE OF IRRADIATION IN PARASITOLOGY**

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Irradiation is a process by which an object is exposed to radiation, which originates from various sources. Irradiation has an important area of usage in parasitology. It is often used to inactivate parasites for the preparation of vaccines, to sterilize the arthropods and to eliminate the parasites and their developmental stages from food. According to the studies, which focused to inactivate parasites for vaccine development, gamma irradiation should be used between150-200 Gy, 350 Gy and 15-175 Gy for Eimeria spp., B. bovis and Plasmodium spp. respectively. Irradiation applying is also being used to sterilize the arthropods like ticks and flies. Irradiation applying to arthropods is frequently used in males and this aimed to suppress spermatogenesis. Although positive results were determined in sterilize arthropod technique, it couldn't decrease chemical fight's popularity. Several parasites in food can be eliminated by different techniques one of which is irradiation applying to foods. It can also protect food quality and improve food safety. Irradiation (150-1000 Gy) successfully applied to foods against Trichinella spiralis, Taenia spp, Toxoplasma gondii, and Cysticercus spp. In conclusion, irradiation technique has a crucial purpose in parasitology. It is needed to determine effect of different ionizing and nonionizing radiation on parasites with further studies.

Keywords: Irradiation, ionizing, parasitology, food





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PREVALENCE OF INTESTINAL PARASITES IN FOOD INDUSTRY WORKERS AND TRADES PEO-PLE IN SHUSHTAR, KHUZESTAN PROVINCE, IRAN

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Today, despite the decrease in incidence and mortality rate of parasitic diseases, human communities are still surrounded by parasitic diseases especially in developing countries like Iran, because of the suitable climatic and geographical conditions. Outbreak of intestinal parasitic diseases is still one of the human public health problems. Thus, particular attention should be paid to shopkeepers and food industry personnel, which are concerned with human food. This issue is the basis for necessity of giving health certificate card to these groups by health responsible. Therefore, we have decided to examine the rate of parasitic infections among shopkeepers and food industry personnel of Shushtar. Materials & Method: Out of 5036 people reffered to Shushtar labs in order to receive Health Cards, 1273 were employees and 3763 shopkeepers. Following the samples collections they were examined by direct method using Lugol's solution. Data collected in 2011 and 2013 were also compared. In this study, 473 individuals were infected with intestinal parasites, which constituted 9.5 percent of the whole population. The highest infection rate of Entamoeba protozoans was 36.3% and of Giardia 32 %. The other parasites were Blastocystis hominis, 17%, Iodamoeba bütschlii 7.1%, Trichomonas hominis 4%, Chilomastix mesnili 2.4%, Hymenolepis nana 1%, and Strongyloides stercoralis 0.2%. Moreover, almost all of studied individuals showed no clinical symptoms. The highest and the lowest season of infection rates were in March-April in spring and October-November in autumn respectively. In spring, due to the nature of the season people has more contact with infected environments and eat unwashed contaminated vegetables and vernal fruits, which in turn might expose them at the parasitic infections. In addition, regular examination and follow up of the industry workers and shopkeepers who are frequently in close contact with people and human foodstuf seems necessary for effective control measures.

Keywords: prevalence, intestinal parasites, Khuzestan

# OCCURRENCE OF CORYNOSOMA CASPICUS (ACANTHOCEPHALA, POLYMORPHIDAE) IN-FECTION IN GASTEOSTEUS ACULEATUS FISH IN SOUTHERN COASTAL OF CASPIAN SEA

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Little information is available on prevalence and density of Corynosoma caspicus in fish, particularly Gasteosteus aculeatus in Iran and all around the world. The aim of present study was to determine the prevalence and intensity of acanthocephalan infection in Babolsar district, southern coastal of Caspian Sea, northern Iran. Matrials & Methods: From September 2012 to August 2014, 360 Gasteosteus aculeatus fish were randomly collected from coastal regions in Babolsar and their intestine and body cavity were examined for worm infections. Of 360 Gasteosteus aculeatus fish, 109 (30.3%) were found to be infected with, at least one corynosoma capsicum, and there was no significant association between sexes and prevalence rate of acanthocephalan. Also, 24 out of 109 (22%) had intense infection rate. The high prevalence and intensity of Corynosoma infection in Gasteosteus aculeatus indicates a stable epizootic status of achathocephalosis in the southern coastal of Caspian Sea.

Keywords: acanthocephalosis, Corynosoma caspicus, Gasteosteus aculeatus, Caspian Sea





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# INVESTIGATION OF THEILERIOSIS IN CATTLES REFERRED TO VETERNAIRY CLINIC OF NOUR-ABAD CITY, LORESTAN PROVINCE

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Theileriosis is an important parasitic disease in cattle. The causal agents of the disease are various species of the genus Theileria. Hyaloma ticks which present in majority of regions in Iran are the vectors of theileriosis.During a 1-year period from 2013-2014, the cattle referred to veternairy clinic of Nourabad city were examined. Diagnosis was performed with observation of syptoms (fever, swelling of lymph nodes and mocusal membranes anemic) and preparing of prepheral blood smear. The result of this study was analyzed with chi square test ( $\chi$ 2). A total of 1634 cattle were examined of which, 280 (17.1%) suspected to have theileriosis. On microscopic examination 172 cases (61.5%) were positive. In positive cattle 36 cases (12.8%) had eosinophilia and 52 cases (18.6%) had sever anemia. In this study no significant association was seen between age and gender (p>0.05). Due to potential of economic losses, careful attention should be directed toward theireriosis, its vectors and routes of the infection.

Keywords: theileriosis, cattle, Lorestan

# COMPARISON OF HEMATOLOGIC INDICES IN PEOPLE INFECTED WITH BLASTOCYSTIS HOM-INIS AND HEALTHY CONTROLS IN AHVAZ, SOUTH WEST OF IRAN

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Blastocystis hominis is a widespread and highly prevalent parasite in humans and many animal hosts in developing countries with increased impact on public health. The aim of this study was to compare hematologic indices in cases with B.hominis infection and healthy controls. From 2001 to 2012, 97600 stool examinations were done in 4 university hospitals. Parasites were observed in 46200 (47.3%) specimens. Of these cases, subjects with complete laboratory investigation (CBC, Ferritin, TIBC, and serum Iron) and B. hominis infection were included in this study as the case group. SPSS version 13.0 was used for analysis. Independent sample-t-Test and Chi-square tests were used for statistical analysis. Result: ESR level was significantly higher in cases with B. hominis infection (P<0.05). CRP level was positive in 1.46% of cases and 0.5% of control which was statistically significant (p < 0.05). Frequency of serum iron < 120 was significantly higher in cases with B. hominis infection compared to controls. Occult blood was positive in 0.93% of cases while it was negative in control group (P<0.05).ESR, CRP, and occult blood were significantly higher in cases infected with B. hominis. It seems further follow-up is essential in B. hominis infected patients.

Keywords: Blastocystis hominis, hemoglobin, serum iron





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#### CASE REPORT OF COENURUS CEREBRALIS IN THE MUSCLE OF A SHEEP FROM MASHHAD

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Coenurus cerebralis infection has been observed as a common and worldwide problem of small ruminants. Dog as definitive host of Taenia multiceps plays an important role in spreading of the disease. Occurrence of coenurosis cysts in brain, spinal cord, muscle and other tissues have been noticed in a wide range of animals including sheep, goats, cattle, horse, buffalo and camel. During meat inspection in abattoir of Mashhad, tumor-like or cyst-like swellings were observed on muscles of femor. In laboratory, macroscopic and microscopic examination was followed by removal of the structures, their dissection and examination under a microscope.In the cysts, multiple semitransparent, fluid-filled protoscolices directly attached to the inner surface of the cyst wall were observed. Then protoscolices become transparent, stained with Carmen-blue and observed under microscope. Pear form protoscolices with suckers and two rows of rostellar hooks were determined. Large and small hooks were measured as 150-170 and 90-130 micrometers respectively. A curvature and swelling were on the handle of large and small hooks, respectively. The appearance of these cysts was consistent with that of coenurus. Although coenurus cysts are mostly located in CNS, more attention should be paid to muscle infection by coenurus in ruminant especially in sheep.

Keywords: sheep, coenurus cerebralis, muscle, Mashhad

PREVALENCE OF HYDATID CYST AND HEPATIC TREMATODES INFECTION IN SLAUGHTERED ANIMALS IN BROJERD

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Hydatid cyst and hepatic trematodiasis are the common parasitic diseases of animals and humans with a worldwide distribution and are accounted for serious health and economic problems in Iran. According to the importance of the subject and necessity for awareness of the status of abovementioned diseases, the current study was performed for determining the prevalence of hydatidosis, fascioliasis, and dicrocoeliasis in slaughtered animals in Brojerd in 2014. This descriptive study analyzed liver and kidney of 37124 cattle and calves, 26354 goats and lambs, 146509 local and non-local sheep and lambs in Borojerd, slaughtered from March 2011 to November 2014 in terms of contamination to hydatid cyst and hepatic trematodes of the Genus Fasciola and Dicrocoelium. Liver and lung of slaughtered animals were exactly examined and in case of observation and confirmation of hydatid cyst, Fasciola and Dicrocoelium, the result was recorded in and analyzed by SPSS software using descriptive and analytical statistics. In this descriptive study, 209987 cattle, sheep and goats were examined of which 3260 (1.55%) animals were contaminated with Fasciola, 2454 (1.17%) with Dicrocoelium and 13329 (6.34%) with hydatid cyst. The findings showed that in the first 8 months of 2014, percentage of contamination with hepatic trematodes and hydatid cyst in 3 studied hosts was more than the previous years. The research also indicated that there is difference between contamination with the studied parasites and hosts on analysis so that the parasitic contamination in goats is less than other slaughtered animals. The research indicated that contamination with hydatid cyst and hepatic trematodes in animals slaughtered in Borojerd is high. Therefore, it is advised to conduct necessary measures for cotrolling the situation and treatment of the infected animals.

Keywords: prevalence, cattle, sheep, goat, *Dicrocoelium*, *Fasciola*, hydatid cyst, Borojerd





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RELATIONSHIP BETWEEN BLASTOCYSTIS HOMINIS INFECTION AND INFLAMATORY BOWEL SYNDROME (IBS) AND COMPARISON OF DIRECT WET MOUNT, CULTURE, TRICHOROM STAINING AND FORMALIN-ETHER METHODS FOR DETECTION OF THE PARASITE

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Blastocystis hominis is an intestinal parasite of humans and animals, which has recently been reported as the causal agent of digestive tract disorders. Inflammatory bowel syndrome is a digestive system disorder with unknown etiology. Some researchers suggested relationship between IBS and B. hominis infection. Therefore, the aim of this study was to evaluate this relationship in a case -control study. In a case-control study, 81 patients with IBS enrolled as case group and 81 patients without IBS but affected with other GI disorders as control group. Stool samples were taken and examined by four methods: direct wet mount, formalin-ether concentration, trichrome staining and culture. Infection rate for B. hominis compared between two groups by chi-square test. A total of 43 (53%) females and 38(47%) males included in each group and, adjusted in terms of age and sex. Infection rate for B. hominis were 45.67 % (37 specimens) in case group compared with 22.22% (28 patients) in control group. There was a significant difference between two groups statistically (P < 0.001, OR = 2.1). The trichrome staining method had the lowest and culture method showed the highest sensitivity to detecting B. hominis in stool samples. The result of this study indicated that, there is significant relationship between infection to Blastocystis hominis and inflammatory bowel syndrome, and the culture method was more sensitive than the other methods for detection of the parasite.

Keywords: Blastocystis hominis, IBS, case-control

GASTROINTESTINAL HELMINTHS OF THE CASPIAN TURTLE, MAUREMYS CASPICA (TES-TUDINES), FROM NORTHERN IRAN

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The Caspian turtle (Mauremys caspica) is a semi-aquatic and adaptable reptile. To date, there are no reports on the parasites of this turtle in Iran. Hence, the current survey was designed to prepare a list of the gastrointestinal helminth parasites of the Caspian turtle in Northern Iran. A total of 34 road-killed individuals (14 males and 20 females) were collected between July 2011 and October 2012 from Mazandaran province, Iran. All parts of gastrointestinal were parasitologically scrutinized and collected specimens were fixed and preserved in 70 % ethanol. Half of the examined Caspian turtles (17) were infected with at least one parasitic helminth. The list of helminths includes three nematodes: Serpinema microcephalum (Camallanidae), Falcaustra armenica (Kathlaniidae), Oxyuridae sp., and one digenean: Telorchis sp. (Telorchiidae). This is the first report of the gastrointestinal helminth parasites of the Caspian turtle in Iran and all helminth species are reported for the first time in Iran.

Keywords: helminth, parasite, Caspian turtle, reptile, Mauremys caspica, Iran





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#### HELMINTH PARASITES OF EASTERN EUROPE-AN HEDGEHOG (ERINACEUS CONCOLOR) IN NORTHERN IRAN

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Recently there is a high tendency among exotic pet owners for keeping hedgehogs. This mammal can transfer some significant zoonotic pathogens to human. Hence, the present study was conducted for the first time to prepare a list of helminth parasites of hedgehogs (Erinaceus concolor) in north of Iran. A total number of 10 (four males and six females) road killed hedgehogs were collected from April to January 2011 in rural areas of Babol city, Mazandaran province, Iran. All of internal organs were scrutinized for helminth burden. The extracted specimens were fixed and preserved in 70% ethanol and then cleared in Lacto-phenol solution. Helminth identification was carried out according to available systematic keys. All the examined hedgehogs (100%) were infected with parasitic helminth as following: two hedgehogs (20%) were infected with Crenosoma striatum, four hedgehogs (40%) harbored Physaloptera clausa, one (10%) host had Hymenolepis erinacei and three (30%) of them were infected with Nephridiacanthus major. This is noteworthy that the current survey is the first report of helminth parasites fauna of Eastern European Hedgehog in Iran.More research is required to perform on unexplored areas of Iran in order to increase our knowledge regarding hedgehog parasitic diseases

**Keywords:** helminth parasite, hedgehog, *Erinaceus concolor*, Babol

THE FIRST REPORT OF HAEMOGREGARINA SPP. INFECTION IN TURTLE (MAUREMYS CAS-PICA) IN IRAN

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Siddall (1995) recognized 19 species of Haemogregarina, all in turtle hosts, and two other genera that parasitize fish, Cyrillia and Desseria. Haemogregarina is a genus of haemoprotozoan parasite mainly in cold-blooded vertebrates. They are unicellular organisms which are parasitic in the red blood cells. In view of the paucity of life cycle studies among reptilian Hemogregarine species, it is likely that Haemogregarina will be found to occur in reptiles other than turtles that have contact with leeches. There is not any report about the Haemogregarina from turtle in Iran. This is the first report of Haemogregarina infection in turtle. Four turtles (Mauremys caspica) from Shiraz area were submitted to parasitology laboratory for diagnosing of infection. Blood samples were collected from infected turtles, then four thin blood smears were immediately prepared for each turtle, air dried, fixed in 100% methanol, and stained with a modified Giemsa stain. Parasitemia was determined by counting the number of parasites observed during examination. All turtles' samples were positive for Haemogregarines, with parasitemia 8 %. These parasites are generally considered non-pathogenic, and have been described in the red blood cells of desert tortoises. They are elongated to fusiform oval organisms found in the red blood cells. Although the size varies, they are larger than the cell's nucleus. The organism stains a basophilic colour and has a surrounding clear zone. It seems that this species is Haemogregarina stepanova and need molecular phylogenetic study.

Keywords: Haemogregarina, Mauremys caspica, Iran





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PCR –BASED SUBTYPING OF BLASTOCYSTIS ISOLATES FROM SYMPTOMATIC AND ASYMP-TOMATIC PATIENTS IN NORTH-WEST OF IRAN

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The pathogenic potential of *Blastocystis hominis* is still controversial. To find the pathogenicity of this organism for determining the genotypic differences among symptomatic and asymptomatic isolates can be useful. The aim of this study was to determine genetic diversity of *Blastocystis hominis* in North West of Iran. 57 isolates from 34 asymptomatic healthy individuals and 23 symptomatic patients were genotyped by polymerase chain reaction using seven pairs of known subtype specific sequence tagged site (STS) primers. Out of 57 isolates, subtype 3 diagnosed predominant (50.87%) followed by ST1 (40.35%) and ST2 (8.77%).By comparing genotypes of *Blastocystis* isolates among asymptomatic group subtype 3 was most dominant (29/34) and all patients in symptomatic group classify as 1 (23/23).

Keywords: Blastocystis hominis, PCR, genotype, Iran.

#### THE PREVALENCE OF MACROSCOPIC CYSTS OF SARCOCYSTIS IN SHEEP SLAUGHTERED IN IS-LAMABAD GHARB, IRAN

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Sarcocystis is an intracellular zoonotic parasite. It is well known that the herbivores are as the intermediate hosts and the carnivores are the final hosts. The most common route of infection in intermediate hosts is ingesting of sporocysts with grass or water. The current study was done in 2012 to determine the prevalence of macroscopic cysts in sheep carcasses slaughtered in Islamabad Gharb to provide some suggestion to reduce the infections. For this purpose, with frequent presence in the industrial slaughterhouse of Islamabad Gharb and accessibility to the data recorded by slaughter inspectors, the prevalence of infection in different seasons was determined. The results showed that 1740, 1750, 1700 and 1703 sheep were slaughtered in spring, summer, autumn and winter respectively in which 0.86 % in spring (15 sheep), 0.68 % in summer (12 sheep), 0.17 % in autumn (3 sheep) and 23% in winter (4 sheep) were infected with macroscopic cysts of Sarcocystis. The prevalence of Sarcocystis infection in 2012 was 0.49 %, which is very low compared to other parts of the country. The infection, could be reduced by control and inspection of the carcass in slaughterhouse and avoidance of placing the contaminated meats for cat and dog.

Keywords: Sarcocystis, cyst, sheep, Islamabad Gharb





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A SURVEY OF ECTOPARASITES OF PERSIAN SQUIRRELS (SCUIRUS ANOMALUS) REFERRED TO THE SMALL ANIMALS CLINICS IN AHVAZ CITY

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Persian squirrel is one of the native rodents of Iran. The Persian squirrel has a long furry tail, which is longer than half of the body. Heads hair and back of the tail is reddish fawn. Red head and tail of Persian squirrel are special features different from other squirrel in Iran. Currently this animal has become one of the favorable pets for Iranian families. In spite of this fact, there is not enough information about the diseases and infections of these animal especially parasitic infections. This study was carried out on 36 Persian squirrel referred to small animals clinics of Ahvaz, to evaluate the rate of ectoparasite infestations. All the squirrels were of Persian squirrel specie. The squirrels were anesthetized or inhibited by thick gloves. Parasites were isolated from the animal's body by using forceps or brushing of body hair in a white tray and stored in 70% ethanol. The specimens were cleared in potassium hydroxide solution and mounted on slides to identify species with a light microscope. In this study, 16 of 36 (44.4%) squirrels were infested with lice. Signs such as weakness and itching were observed in infected animals. Parasites accumulate more in the groin, axilla and back of the neck. A total of 78 lice were recovered from 16 infested examined squirrels. The lice were identified following the description of Johnson. All the lice were identified as Neohaematopinus spp.Due to high prevalence of Neohaematopinus spp. infection among Persian squirrels, pet owners should be informed about the importance and methods for control and prevention of ectoparasite infestations, especially lice. No danger threatens the health of pet owners, due to the host specificity of lice.

Keywords: Persian squirrels, Neohaematopinus spp., Ahvaz

#### EFFECT OF GLYCYRRHIZA GLABRA L. AND PRIMULA AQUALIS L. EXTRACTS ON REMOV-ING PARASITE OVA FROM VEGETABLE PARSLEY

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Vegetables are a major constituent of human diet and a potential source of food-borne diseases especially parasitic infections. Washing is an important step to reduce infection burden and washing agents are important as well. This research was designed to investigate the removal effect of Glycyrrhiza glabra and Primula aqualis aqueous extractions on parasite disinfection from parsley in comparison to commercial detergents in Sari city. Roots aqueous extract (5, 10, 15, and 20 percent concentrations) was prepared from Glycyrrhiza glabra and Primula aqualis by maceration method. 100 g of parsley vegetable sample was randomly taken from market. The sample was placed in contact with aqueous extract for 0 and 15 minutes contact time. Then, the effluent was centrifuged, finally, quantity and variety of isolated parasite was counted by Mac- Master counting slid, (0.3mm). Result: At 0 contact time, the maximum parasite removal observed in Glycyrrhiza glabra 20%. At 15 min. contact time, the maximum parasite removal observed in Primula aqualis 15% that included 382 flagellates, 721 Ciliates, 33 Ascaris eggs out of 1136 parasites. Variety and number of parasites were more on the extraction at 15 % concentration in two contact times- 0 and 15 min. The result of this study obviously showed that plants extracts could be used as natural washing detergent and regarding to availability and ease of use, they could be replaced for commercial detergents.

Keywords: *Glycyrrhiza glabra L.*, *Primula aqualis L.*, vegetable washing, parsley, parasite removal





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TENUICOLLIS FROM SHEEP BY SEQUENCING OF MITOCHONDRIAL CYTOCHROME C OXI-DASE SUBUNIT 1 (COX1) GENE IN CHABAHAR, ZAHEDAN PROVINCE

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Cysticercus tenuicollis is the larval stage of the canine tapeworm Taenia hydatigena, which is prevalent in domestic and wild ruminants. T. hydatigena is one of the most prevalent cestodes in dogs in Iran. Metacestodes are in liver of intermediate host. The aim of this study was to provide molecular characterization of the sheep isolates of C. tenuicollis by PCR amplification partial sequencing of COX1 gene. C. tenuicollis bladders were collected from liver of sheep during routine veterinary inspection in abattoir of Chabahar, Zahedan province, Iran. Two samples were transported to Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences. Following DNA extraction, fragment of cytochrome C oxidase 1 (COX1) gene was amplified by PCR. After sequencing of amplicons, they were edited and aligned, using Chromas software. Sequences analysis was undertaken by BLAST algorithms and databases from GenBank. Result: The amplified fragment size was approximately 446 bp. Partial sequence of COX1 gene was corresponding with C. tenuicollis. The isolates were different with each other in one nucleotide. According to the alignment results; two isolates had 99% homology with C. enuicollis in GenBank with accession number DQ995656 and AB792722, respectively. In order to study probable difference between isolates in the study area, sequencing of more isolates from different hosts is needed.

**Keywords:** Cysticercus tenuicollis, *Taenia hydatigena*, cytochrome C oxidase 1 gene

#### EPIDEMIOLOGICAL ASPECTS OF ENTEROBIAS-IS AMONG PRESCHOOL CHILDREN IN MA-ZANDARAN PROVINCE

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The aim of this study was to determine the prevalence of Enterobiasis and the association between clinical features, potential risk factors among preschool children in Mazandaran region. In this cross - sectional study 653 preschool children randomly assigned to the seven districts and evaluated using Graham technique for oxyuriasis. Demographic data with questionnaire-designed questions were collected. SPSS version 17 and  $\chi^2$  test for statistical analysis was used. Of 653 children, 127 (19.4 %) were infected with Oxyuris- 52 (40.9%) were male and 75 (59.1 %) were female. No relationship was found between infestation and gender. The highest infection rate was observed in the city of Neka (61.4 %) and the lowest infection rate in Tonkabon and kyasar (6.1%). The relationship between infestation and residence, parental occupation, parental education, number of household members, changing underwear, sterilizing linen clothes, bathing daily, boiling or ironing clothes were significant. The relationship between infestation and clinical signs was also significant.

**Keywords:** oxyuriasis, preschool children, province, epidemiology





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#### EFFECT OF HYDROALCHOLIC EXTRACT OF POMEGRANATE ROOT ON TRICHOMONAS VAGINALIS (INVITRO)

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Trichomoniasis is one of the most common sexually transmitted disease all around the world caused by Trichomonas vaginalis. Considering the increasing prevalence of the disease, known side effects of metronidazole and increasing reports of resistance to this medicine, herbal therapy has been noticed in recent decades for reducing the side effects. The aim of this study was to investigate the effect of hydroalcholic extract of pomegranate root on Trichomonas vaginalis in vitro.In this experimental study the numbers of parasites were 500000 per ml, by neobar slide at the start of the test. Positive control was metronidazole (concentration =5 mg/mlat the beginning of the test) and negative control was medium containing parasite. At first, hydroalcholic extract of pomegranate root was prepared. Trichomonas vaginalis removed from women referred to health centers and were maintaind by passage in TYM culture and were confirmed by direct observation; then added to 24-well plates and degree of influence of the concentration of (50,100, 200, 400, 800, 1600 microgr / ml) was studied completely unconscious during 24, 48, 72 hours in aspects of numbers (counting by slide), viability and mobility (with Trypan blue color). Checking the negative control slide was counted by Neobar slide. The number of parasites was 500000 in negative control, which was the same after 72 hours. Cytotoxicity effect of parasite was observed in all of the concentration of pomegranate root but the best result obtained in 800 mic/ml, which reduced the numbers of parasites to 40000, and 1600 mic/ml which destroyed all the live parasite after 72 hours.Extract of pomeganate root as a herbal drug is effective on Trichomonas vaginalis.

Keywords: Trichomonas vaginalis, extract, pomegranate, modified TYM Medium PREVALENCE OF BLASTOCYSTIS HOMINIS IN THE CITY OF TABRIZ, IN 2013-2014

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Blastocystis hominis, protozoan parasite, anaerobic and zoonosis are found in the large intestine of humans and most other vertebrates. It has a worldwide distribution and is repeatedly separated in parasitology laboratories. Given the relatively high prevalence of Blastocystis and emphasis on its pathogencity, especially in people with impaired immune systems in recent researches, the following study aimed to determine the prevalence of Blastocystis in Tabriz city from April 2013 to December 2014. In this study, 53,450 samples collected from 17,817 women and 35,633 men with the age ranging from 25 to 47 years old, referred to the central laboratory of the province. From 53,450 patients 649 (1.21%) were infected- 0.81% among men and 0.4% among women. The most common symptoms in patients with Blastocystis was abdominal pain, nausea and anorexia. The results showed Blastocystosis as the most common intestinal parasitic infections creating many problems for the patients. Therefore, the use of appropriate diagnostic procedures in laboratories can be effective in early and correct diagnosis and will play an important role in improvement of public health.

Keywords: Blastocystis hominis, prevalence, Tabriz





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FREQUENCY OF DIENTAMOEBA FRAGILIS AMONG 2 TO 6 YEARS OLD CHILDREN IN ARAK KINDERGARTENS, IRAN

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Dientamoeba fragilis is a pathogenic protozoan of human intestinal tract, which usually causes diarrhea. Studies show that children are more susceptible to D. fragilis infection and clinical symptoms are more likely than adults. The purpose of this study was to determine the prevalence of D. fragilis among 2-6 years old children in kindergartens of Arak. In a descriptive cross-sectional study, 333 triple fecal samples were collected from 2-6 years old children in 4 districts of Arak city. Fecal samples were investigated by microscopic method, culture in horse serum and Loffler's media, formal-ether method, trichrome staining and PCR using 5.8S rRNA gene. Of 333 children, 172 cases (51.7%) were male and 161 (48.3%) were female. Dientamoeba was not found by microscopic and formalin-ether methods. Eleven cases (3.3%) were detected with culture methods. Trichrome staining revealed 13 (3.9%) Dientamoeba. Amplification of 5.8SrRNA gene showed 101 (30.3%) Dientamoeba infection. No significant statistical differences was observed between frequency of Dientamoeba and age groups and sex. The trichrome staining is an appropriate method for detection of this parasite accompany with other methods. Polymerase chain reaction (PCR) is a sensitive and specific method for detection of Dientamoeba. As helminth eggs were not observed in the studies individuals and Dientamoeba existed with other protozoan parasites, this hypothesis supports that the transmission route of this parasite is fecal- oral.

Keywords: *Dientamoeba fragilis*, frequency, culture, staining, polymerase chain reaction, Arak

#### THE RELATIVE FREQUENCY OF INTESTINAL PARASITIC INFECTION IN PATIENTS RE-FERRED TO THE TWO MAJOR LABORATORIES IN RASHT, GUILAN, IRAN DURING A 6-MONTH PERIOD IN 2014

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The prevalence of intestinal parasitic infections is an important factor to assess the public health status, so a regular survey is essential to control parasitic disorders. This article is aimed to study the relative frequency of intestinal parasitic infection in patients referred to the two major laboratories in the municipality of Rasht (with the population of 918445 capita) during a six-month period from May to October in 2014. A cross-sectional study based on the results of stool examination was carried out using the variables related to age, gender and the type of identified parasites. The routine methods of stool examinations in both laboratories were wet mount, floatation, formalin-ether sedimentation, agar plate and scotch tape techniques. Out of 12956 stool samples, 228 specimens (1.75%) were infected including 139 male (60.96%) and 89 female (39.03%). The relative frequency of all diagnosed parasites in stool tests referred to the whole study population were Giardia lamblia (113 samples, 0.87%), Entamoeba coli (66 samples, 0.50%), Strongyloides stercoralis (29 samples 0.22%), Blastocystis hominis (10 samples, 0.7%), Iodameoba butschlii (5 sample, 0.03%), Enterobius vermicularis (3 samples, 0.02%) and Entamoeba histolytica (2 samples, 0.01%), respectively. Out of the positive stool samples the two age groups of under, 10 (with 60 positive stool sample, 26.31%) and above 50 years old (with 64 positive stool samples, 27.07%) were the most infected. The results indicate that Entamoeba coli, which is a nonpathogenic protozoa, and Giardia lamblia have the most frequency among infected patients. Out of the positive stool tests the two age groups of under 10 and above 50 years old indicated the most parasitic infection and the majority of them were male. Therefore, appropriate control method is needed to follow up sanitation trends and prevention of intestinal parasitic infections especially Giardiasis and two most infected age groups.

Keywords: intestinal parasite, relative frequency, Rasht.





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EPIDEMIOLOGICAL STUDY OF ZOONOTIC BALANTIDIUM COLI IN WILD BOARS IN BU-SHEHR PROVINCE IN 2014

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Wild boars, Sus Scrofa, live as dense populations in forests in south and south-west, north and northeast of Iran. These animals may act as a potential threat for some people, including farmers and hunters. So far, prevalence of zoonotic Balantidium coli in wild boars has not been evaluated in Bushehr. The current study aimed to assess the prevalence of this zoonotic protozoan in wild boars in Deylam district of Bushehr province. This cross-sectional study was conducted on 25 cross bred hunted boars in Deylam district from May to October 2014. After carcasses autopsy, stool samples of colon extremity (rectum) was collected in 5% formalin, Bouin's solution, SAF and PVA fixatives. The samples were examined by different methods including wet direct smear, lugol staining, trichrome staining and formalin ethyl acetate concentration technique. From 25 hunted boars, 11 (44%) were male and 14 (56%) were female. Mean weight of the boars was 131 kg (45-275) and mean age of the animals was 3.4 years (1-9 years). Infection with Balantidium coli was detected in 12 (48%) of 25 studied wild boars. Infection in male boars was 54.5% and among females was 42.8%. Rate of Balantidium coli infection in wild boars in Deylam district in Bushehr province is considerable. To prevent the local residents especially farmers and hunters, it is necessary to educate people about the disease and the way that the infection can be prevented.

Keywords: Balantidium coli, epidemiology, Sus Scrofa, Iran

ASSESSMENT OF PARASITIC CONTAMINATION OF THE VEGETABLES SOLD IN RASH, IRAN

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Nowadays, concern to take healthy food made people more interested in consuming vegetables. Vegetables can be a potential source for parasitic infections. This study aimed to assess parasitic infections of the vegetables sold in Rasht markets. A total of 342 samples from 12 kinds of vegetables were purchased from Rasht markets and examined during years 2011 to 2014. Approximate weight of each sample was 200 g. Each specimen separately was washed and washings left to settle over night. Supernatant were discarded and the remainings were centrifuged. The sediments were examined through light microscopy and pathogenic parasites were diagnosed. Statistical analysis performed using chi-squre ( $\chi 2$ ) and Fischer's Exact Test by SPSS.21 soft ware. Pathogenic parasites were diagnosed in 4.1 % of the vegetable samples. The identified parasites were consisted of Hook worm (0.9%), Toxocara sp. (0.3%), Trichuris sp. (0.9%), Trichostrongylus sp. (0.9%), Strongyloides stercoralis (0.3%), Acanthamoeba sp. (0.6%), Naegleria sp. (0.3%), and Diploscapter sp. (0.3%). Various vegetables were similar in terms of parasitic infection (P=0.357). Parasitic contaminations of vegetables based on season (P=0.474) and year (P=0.344) did not show any significant differences. Vegetables cultivated inside the Guilan province were more infected than those grown out side (P= 0.006). Free-living nematods were found in 65.8% of the specimens.Despite the existence of appropriate climates for propagation of human pathogenic parasites in Guilan province, frequency of parasitic contamination of the common vegetables in Rasht was less than reported figures of similar studies from other places in Iran.

Keywords: vegetables, parasitic contamination, Rasht, Iran.





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#### COCCIDIAN INFECTION IN WHITE-EARED BULBUL (PYCNONOTUS LEUCOTIS)

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The white-eared bulbul (Pycnonotus leucotis) is a member of the bulbul family found in southern Iran and Tehran. Coccidiosis is an intestinal disease of birds caused by various species of protozoan parasites within the genus Eimeria (Apicomplexa. Eimeriorina. Eimeriidae). This disease has a major economic impact to growers and to the poultry industry worldwide. Eimeria spp. are prevalent monoxenous and host specific parasitis. Coccidia in Passeriformes is rare infection and only six species are recognized. This survey aimed tostudy the prevalence of Eimeria spp. oocysts in white-eared bulbuls in Ahvaz. From November 2012 up to June 2013, 60 whiteeared bulbuls were captured around poultry farms of Ahvaz city, Southwestern of Iran, and gradually transferred to avian diseases section, Faculty of Veterinary Medicine of Ahwaz. After clinical examination of each bulbul, they were euthanized and fecal samples were collected from the small and large intestines. Then samples were examined by a claytonlane method. We could not observe any clinical signs in bulbuls. The study of collected samples indicated that 29 of 60 (48.3%) bulbuls were infected by Eimeria spp. with different severity. Coccidiosis is probably not a problem in wild populations because the hosts are widely distributed and not confined to limited areas where the oocysts concentration can increase in the environment to the point that the host would ingest large numbers of oocysts. However, distribution of oocysts would be more difficult in the natural setting.

Keywords: coccidiosis, white-eared bulbul, Ahvaz

#### PREVALENCE OF DICROCOELIUM DENDRITI-CUM INFECTION IN SHEEP IN KHUZESTAN PROVINCE BY AN IN HOUSE ELISA

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Dicrocoeliasis caused by Dicrocoelium dendriticum is one of the most important disease in ruminant specially sheep. Adult worms live in gall bladder and biliary ducts causes huge amounts of injury in infected animals, lead to seize of infected livers in slaughterhouses. Due to the importance of epidemiologic studies and the fact that current parasitological diagnosis of the parasite for identification of the early infection have some problems, procedures such as serologic methods based on suitable antigen are useful tools. Thus this study has been done to evaluate the epidemiology of D. dendriticum by using indirect ELISA in Khuzestan province.500 blood samples were collected from sheep in different cities of Khuzestan and Sera was separated. After determination of somatic protein antigen by using Bradford's method, ELISA was set up by checker board examination and indirect ELISA was performed on sera of collected samples. According to our results, total prevalence of Dicrocoeliasis in Khuzestan was 3.6% (3.5% in females and 4.7% in males). Fisher's test showed that this difference is not statistically significant (P> 0.05). Ratio of infection in males was 1.35 in compare to females. Since our study revealed low prevalence of D. dendriticum, we can conclude that probably sheep has not important role in epidemiology of the parasite in this area.

Keywords: Dicrocoelium dendriticum, sheep, prevalence, Iran





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TABRIZ CITY USING MITOCHONDRIAL CYTO-CHROME OXIDASE1 (COX1) GENE BY PCR-SEQUENCING METHOD

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Enterobius vermicularis is one of the most prevalent parasitic helminths, particularly in children. The most specific diagnostic method is Scotch tape (Graham method). According to the studies, three different genotypes (A, B and C) were revealed from human and chimpanzee. Regarding to the prevalence and public health importance of the disease and role of the parasite genotyping in prevention, control and treatment, further studies are needed to determine genotypes of E. vermicularis. This study was conducted to investigate the existence and distribution of different E. vermicularis genotypes based on mitochondrial cytochrome c oxidase subunit 1 (cox1) in Tabriz. 45 positive scotch tape samples of E. vermicularis were collected from various areas of Tabriz. After DNA extraction, the targeted DNA region was amplified for the mitochondrial cytochrome c oxidase 1(cox1) gene by nested PCR method. All amplicons were sequenced and then analyzed by specific phylogenetic software. In this study, there obtained two sequence types genotype B namely B1 and B2. Twenty samples were divided in B1 sequences (44%) and twenty five samples in the B2 sequences (56%).Based on the results of this study, which was performed for the first time in Iran, B genotype of E. vermicularis is the human genotype of this nematode in Tabriz. B1 sequences were different with sequences registered in GenBank while B2 sequences were similar to some of the sequences registered in GenBank.

**Keywords:** *Enterobius vermicularis*, cytochrome c oxidase 1 (cox1), genetic classification, Tabriz

#### GENOTYPING OF ENTEROBIUS VERMICULARIS FROM THE INFECTED HUMAN SAMPLES IN THE INTESTINAL PARASITIC INFECTION RATE IN AHWAZ IN 2013-2014

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Intestinal parasitic infections are still a major health problem in developing countries. The aim of this study was to investigate the frequency of intestinal parasites in Ahwaz in 2013-2014. Stool samples of 2882 individuals referred to a hospital in Ahwaz were collected and examined macroscopically for any helminthes or proglottids and microscopically for any ova, larva, cyst or trophozoites. The results were analyzed using SPSS version 19 and Chi-square test. From 2882 cases 4.6% were infected with intestinal parasites:2.5% with Giardia lamblia, 0.8% Blastocystis hominis, 0.6% Entamoeba histolytica/dispar, 0.3% Iodamoeba butschelii, 0.2% Entamoeba coli and Trichomonas hominis, and Enterobius vermicularis 0.1% for each. Maximum infection rate was found in spring, and minimum rate in winter with intestinal parasites. The results showed a significant relation between the prevalence of intestinal parasites and seasons of year.

Keywords: intestinal parasites, frequency, Ahwaz, Iran.





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#### EPIDEMIOLOGIC AND PREDISPOSING FACTORS OF SCORPION STINGING IN BAGHMALEK, KHUZESTAN PROVINCE IN 10 YEARS

#### <u>Komeil Mazhab-jafari</u><sup>1</sup>, Ali Asghar Valipour<sup>2</sup>,Azimeh Karimyan<sup>3</sup>,Dariush Bahrami<sup>4</sup>, Firooz Shahrivar<sup>4</sup>,Somaye Azimi<sup>5</sup>

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Scorpion stinging is one of the public health problem throughout the world. Due to the climate variability of the country, various species of scorpions including the dangerous types are present in Iran. Baghmalek, located in north east of Khuzestan province, has appropriate climatic conditions for scorpion living. The aim of the present study was to study scorpion stinging from various aspects in this town. The cross sectional study has been done since 2002 till 2011 in Baghmalek town. All of the scorpion stinging cases were inspected and treated and then a survey was completed consisted of demographic, epidemiologic and clinical information. Statical analysis was done by SPSS and chi-square test. 8675 cases of scorpion stinging were recorded with the highest cases reported in spring and summer. The most common time of scorpion stinging was 12-6 a.m, and the most stung organ was legs and hands. 50% of cases were in people who slept outside and on the ground. In 20% of cases there was brushwood and fire wood around the scorpion stung person. Most of the scorpion-stung patients treated and only 12 persons were died.Regarding to the common scorpion stinging in this region, precautionary measures and promoting education programs should be undertaken for susceptable people to avoid such problems.

**Keywords:** scorpion stinging, epidemiology, demographic, Baghmalek, Khuzestan.

#### EPIDEMIOLOGY OF CUTANEOUS LEISHMANIA-SIS IN BAGHMALEK TOWN IN KHUZESTAN PROVINCE SINCE 2002 TO 2011

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Leishmaniasis is among the ten most important tropical diseases which WHO emphasis on its investigation and supports its related researches. Leishmaniasis is endemic in Iran and because of the increasing its prevalence rates in recent years, this study was performed to evaluate the epidemiologic aspects of cutaneous leishmaniasis in Baghmalek town, north east of Khuzestan province. This cross-sectional study was done on the cases diagnosed as positive according to the clinical symptoms, biopsy and observation of parasite in stained slides. Data concerning age, sex, residence place, type of leishmaniasis, number and location of wounds and diagnostic keys are gathered and analyzed by SPSS. Results showed that of 114 infected cases of cutaneous leishmaniasis 57% were men and 43% women. The prevalence rate was 10 in 100,000 and the infection was most common in children under 5 years old. 81.6% of patients had no history of travel or residence out of the town in one year before being infected.Considering the fact that most cases of the disease have occurred in the city, Baghmalek town is one of the active endemic places in the country.

Keywords: cutaneous leishmaniasis, epidemiology, Baghmalek, Khuzestan





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 ONTAMINATION OF CYPRINUS CARPIO WITH
 EFFEC

# LERNEA IN CHAHNIMEH, ZABOL

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Breeding all kinds of aquatic animals to supply the animal protein is of particular importance in many countries. The aquaculture industry in Iran also has been developed rather favorably over the last decade. In many parts of Iran, Cyprinus carpio is reared in ponds. There are many Cyprinus carpio rearing ponds in Sistan, Iran. However, main sources of supplying Cyprinus carpio in Sistan are Hamoon Lagoon and Chahnimeh. One of the ectoparasites of Cyprinus carpio is the lernaea that causes lesions in the fish and sometimes kills them. A total of 100 fish (Cyprinus carpio), caught from Chahnimeh, were purchased in the market and studied for lernea. The fish were divided to 3 weight groups of 150-200 g, 200-300 g, and 300-400 g. The external surfaces of the fish body were examined and lernea was removed from contaminated fish. The number of parasites on the surface of the fish and the contaminated organ were recorded. Of the 100 fish, 21 were contaminated with lernea, of which 11 were 150-200 g, 9 were 200-300 g and 1 was 300-400 g. The maximum and minimum number of parasites removed from a fish was 9 and 1, respectively. The most contaminated organ was the surface of the fish body. The fish contaminated with lernaea are weaker than other fish and are easily hunted by fisheating birds. Moreover, the contaminated fish have scars on their body that discourage people from buying them. The presence of parasite in the fins and eyes of the fish cause imbalance in the fish and reduces its mobility. Given that this parasite can easily reproduce, it is recommended that contaminated fish not be transferred to other fish pools. It is recommended not to keep the contaminated fish with other fish in the ponds and to disinfect the contaminated ponds using anti-parasitic medications.

Keywords: Cyprinus carpio, Zabol, Chahnimeh,

#### EFFECTS OF TWO ANTIGENS OF HYDATID CYST ON THE GROWTH OF MELANOMA CAN-CER IN C57/BLACK MICE

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Hydatid cyst is the larval stage of *Echinococcus granulosus*. In previous studies inhibitory effect of this parasite on cancer cell growth in culture medium has been shown. In this study effect of hydatid cyst antigens on tumor growth in experimental animals has been investigated. Two antigens of hydatid cyst including protoscolices excretory secretory antigen and hydatid fluid absorbed on alum as adjuvant were injected to two groups of C57/black mice as case groups. Control groups were injected with only saline and alum. All mice then were injected with melanoma cells. Both antigens reduced the tumor size in mice in case groups. The difference of tumor size in mice in case groups and control group was statistically significant.In conclusion, anti-tumor effect of hydatid cyst antigens may be related to antigenic similarities which exist between hydatid cyst and cancer cells.

Keywords: Hydatid cyst, antigen, cancer, melanoma





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CONTAMINATION OF SCHIZOCYPRIS ALTI-DORSALIS WITH LERNEA IN CHAHNIMEH, ZABOL

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Schizocypris altidorsalis is a local valuable fish from Hamoon lagoon and Chahnimeh in Zabol. Given the economic value of this fish for the locals, parasites that hurt and devalue the fish are particularly important. Learnea is an important foreign parasite for fish, which causes lesions on the fish body. Incurring huge losses, learnea is one of the most important parasites for freshwater fish.A total of 100 fish (Schizocypris altidorsalis), caught from Chahnimeh, were purchased in the market and studied for lernea. The fish were divided to 3 weight groups of 80-100 g, 100-150 g, and 150-200 g. The external surfaces of the fish body were examined and lernea was removed from contaminated fish. The number of parasites on the surface of the fish and the contaminated organ were recorded. Of the 100 fish, 86 were contaminated with lernea, of which 53 were 80-100 g, 22 were 100-150 g and 11 were 150-200 g. The maximum and minimum number of parasites removed from a fish were 7 and 2, respectively. The most contaminated organ was the surface of the fish body. Lernea has been found in many freshwater fish in Iran, of which aquarium fish are the most important ones. The most frequent location of the parasite was the fish skin, which reduces the market value of the fish. The parasite causes pathologic lesions and reduce the fish's growth. The presence of parasite in the fins and eyes of the fish cause imbalance in the fish and reduces its mobility. Contaminated fish have a certain appearance and people do not purchase them. Given that this parasite can easily reproduce, it is recommended that contaminated fish not be transferred to other fish pools.

Keywords: Schizocypris altidoralis, parasite, Chahnimeh, Zabol

PREVALENCE OF INTESTINAL PARASITES IN RESIDENTS OF ROUDEHEN, TEHRAN PROV-INCE, IRAN

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Human intestinal parasites remain as a major public health concern globally. Studies show that intestinal parasitic infections are still common in some parts of Iran. Due to the lack of the epidemiologic picture of parasitic intestinal infections in Roudehen, this study was conducted to investigat the prevalence of intestinal parasites in residents in this area. In a cross-sectional study, 561 triple fecal samples were randomly collected from the general population of Roudehen city, from June to December 2014. Fecal specimens were transferred to the research laboratory of the Department of Parasitology and Mycology, Iran University of Medical Sciences. The specimens were investigated by formalin-ether concentration method. Of the 561 people enrolled in the study, 42.2% were male and 57.8% were female. Microscopic examination showed 136 (24.2%) of participants infected with at least one gastrointestinal parasites. Prevalence of intestinal parasites in the studied population was as follows: from the enteric protozoan, 114 (20.3%) Blastocystis sp., 25 (4.5%) Entamoeba coli, 7 (1.2%) Giardia lamblia, 1 (0.2%) four-nuclei Entamoeba cysts, 4 (0.7%) Iodamoeba butschlii, 1 (0.2%) Chilomastix mesnili, and only one case of intestinal worm. Eggs of Enterobius vermicularis (0.2%) were reported in the population. Compared to recent studies conducted in other cities throughout the country, the prevalence of intestinal parasites, especially parasitic helminth in Roudehen is also low. Blastocystis sp. was the most prevalent among intestinal parasites observed in this area. Given that most of these parasites are transmitted via the fecal-oral route, the individual health education can play an important role in improving the health and preventing the risk of transmission of disease to people living in this area.

Keywords: intestinal parasites, Formalin-ether method, Roudehen





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#### PREVALENCE OF INTESTINAL PARASITIC IN-FECTIONS IN CHILDREN REFERRED TO MOFID HOSPITAL, TEHRAN

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Today the infectious diseases create many problems in various parts of the world. However the injuries and the damages caused by these diseases mostly affect developing countries. Parasitic diseases in creating social and economic damages approximately equals to some disease such as tuberculosis, sexually transmitted diseases, vaccine preventable diseases and acute respiratory infections. Parasitic infections are more common and has greater importance in children and their early diagnosis and treatment improves growth and development of the children. In this study 250 stool samples were collected from children reffered to Mofid hospital. The samples examined with formalin-ether concentration method of and studied under light microscope. Of 250 studied inviduals 165 (66.25%) were male and 85 (34.2%) female among which 43(17.16%) cases were positive for intestinal parasites. Rate of contamination to Blastocystis hominis was (12.5%), Giardia lamblia (3.40%), Iodamoeba butschlii (1.2%), Enterobius vermicularis (0.04%) and Entamoeba histolytica (0.02%). No significant difference was found between infection rate genders. Conclusions: Results of this study showed that the infection rate of intestinal pathogenic protozoa, especially Blastocystis hominis, in children were more than intestinal pathogenic worms which may be due to lower personal hygiesn and existence of autoinfection process in children and also the simple cycle of parasite transmission among people.

Keywords: intestinal parasites, children, autoinfection, Tehran

#### A CASE REPORT OF ACUTE GASTROENTERITIS DUE TO LEECH BITE

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Leeches are a group of roundworms that belong to Hirudina order. They live in freshwater, soil and wetlands. Some leeches are predators and feed on worms, insect's larvae, snails and other invertebrates whereas some others are bloodthirsty and feed on invertebrates and vertebrates. Although leeches can have important therapeutic traits, sometimes cause serious problems in humans and animals. In the present report, a 22 year old woman living in Tabriz admitted to infectious diseases ward of Sina hospital with diarrhea, nausea and gastroenteritis symptoms. After clinical and parasitological experiments, leeches were confirmed in the patient's gastrointestinal secretions.

Keywords: leeches, gastroenteritis, Iran





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#### COMPARISON OF DISINFECTION ACTIVITIES OF NICOTINE WITH COPPER SULPHATE IN WATER CONTAINING LIMNATIS NILOTICA

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Most leeches are bloodthirsty and suck the blood of their prey. In this study, we investigated the potential use of nicotine in controlling waters polluted by leech. The nicotine and copper sulphate LC50 values were determined following 30 min exposure. The anti parasitic effect of nicotine was also compared with that of copper sulphate as positive control. The anti-leech effect of nicotine was evaluated against L. nilotica in which the number of dead and alive leeches in each utensil was counted for 30 minutes. The positive control group was copper sulphate and the negative control was distilled water. Our data showed that the LD50 value for nicotine was  $6 \times 103$  ppm with mean death time of  $1.25 \pm 0.45$  min, while the LD50 value for copper sulphate was 637×102 ppm with mean death time of 12.00±3.69 min.Based on the obtained results; nicotine is highly effective on leeches and might be used for disinfection purposes.

Keywords: disinfection assay, L. nilotica, nicotine, copper sulphate, LC50

### INTERNAL CONTAMINATION WITH LEECH IN A TURKEY (MELEAGRIS GALLOPAVO)

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Leech enters to mouth and nose through water. Nose and nasopharynx mucosa are the most preferred place for leech attachment with epistaxis and respiratory distress symptoms, but rarely it may stick deeper to trachea or esophagus, causing hematemesis, hemoptysis and severe respiratory distress. Leech infestation can cause gastrointestinal, respiratory and genital bleeding in rare cases. Various animals such as ruminants, single-toed and carnivores are infected with leeches. In May 1393, a two years old turkey infected following the contaminated drinking water referred to veterinarian with respiratory distress symptoms, anxiety, bleeding from the mouth in Down Maze-Abdali village located at 17 kilometers far from Dehloran city of Ilam province in the West of Iran. After physical observations, a moving dark green particle was seen. Limnatis nilotica were detected after separation from the oral cavity of turkey. In areas where springs and flooded waters are infested with leeches, respiratory distress and oral cavity bleeding should be regarded. Drinking untreated and contaminated waters should be avoided.

Keywords: turkey, leeches, *Limnatis nilotica*, respiratory distress, oral cavity bleeding, Iran





Proceedings of 2nd International and 9th National Congress of Parasitology and Parasitic Diseases of Iran (NICOPA 9)

Guilan, Iran; 20-22 May 2015HELMINTH PARASITES INFECTION OF POUL-<br/>TRY IN HAMEDAN PROVINCE, WESTERN IRAN,THE PRE<br/>IN BAQIN

IN 2014

#### <u>Marzieh Asadi</u><sup>1</sup>, Masoud Hamzehkhani<sup>2</sup>, Mohammad Fallah<sup>3</sup>

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Due to the way of nutrition and mechanisms of contact with their environment, poultry are at risk of different parasites. The present study aimed to determine the prevalence of helminth parasitic infections of poultry in Hamedan province, in 2014. This study was conducted on 46 samples of intestinal contents of poultry including 33 poultry and 13 rooster. All the worms collected and stored in 70% ethanol with 5% glycerol and then were transparated using lactophenol and microscopically examined after staining with Carmine acid technique. Of the 46 samples of intestinal contents of studied poultry, 23 (48.93%) were found to be infected with intestinal cestode. Raillietina echinobothrida was the most frequently detected parasite with prevalence of 29.78%, followed by Raillietina tetragona (19.14%). Infection in poultry, particularly in laying poultry was more than rooster. The results of this study showed a high prevalence of intestinal cestodes in Hamedan province. Therefore, it is necessary to prevent and control infections by anti helminthics and reform in poultry growing.

Keywords: helminth parasites, poultry, Hamedan

THE PREVALENCE OF INTESTINAL PARASITES IN BAQIYATALLAH HOSPITAL, TEHRAN, IRAN

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Parasitic infections are among important health problems all over the world especially in developing countries. According to the World Health Organization report, more than twothirds of the world population are infected with at least one parasite. Intestinal parasites, particularly Giardia, have been reported to be common. Considering the epidemiological importance of parasitological diseases and necessity of evaluation of parasites prevalence in different areas and also due to the large number of patients referring to Baqiyatallah hospital, current study was conducted to determine the prevalence of intestinal parasites including protozoa and helminths in patients admitted to the laboratories of Baqiyatallah hospital. In this retrospective study all patients, suspected to have intestinal parasitic infections, (70978) referred to the laboratories of Baqiyatallah hospital during September 23, 2010 to September 23, 2014, were evaluated. From 70978 patients, 2.49%, 0.15 %, 0.16%, 0.04%, 0.55%, 0.26%, 0.003%, 0.013%, 0.001%, 0.006%, 0.004 and 0.004% were infected with Blastocystis hominis, Entamoeba coli, Entamoeba histolytica/dispar, Chilomastix mesnili, Giardia lamblia, Endolimax nana, Trichomonas hominis, Iodamoeba butschlii, Taenia Sp., Enterobius vermicularis, Strongyloides stercoralis and Hymenolepis nana, respectively. The result of present study showed "Blastocystis hominis" as the most prevalent intestinal parasite. More attention should be directed towards the improving the quality of laboratory and clinical diagnosis of this protozoa.

**Keywords:** intestinal parasites, protozoa, helminths, Baqiyatallah Hospital





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 COMPARATIVE EFFICACY OF NUTRIENT AGAR
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 CONCENTRATION METHODS IN THE LABORA
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CONCENTRATION METHODS IN THE LABORA-TORY DIAGNOSIS OF HUMAN TRICHOSTRON-GYLIASIS

# <u>Bahareh Najmi</u><sup>1</sup>, Mostafa Hosseini<sup>2</sup>, Iraj Mobedi<sup>2</sup>, Bahareh Kamranrashani<sup>3</sup>, Eshrat Beigom Kia<sup>2</sup>

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Trichostrongyliasis is a zoonotic disease caused by Trichostrongylus species. This nematode is common among domestic and wild herbivores and can also infect human. Low rate infections may be missed during laboratory diagnosis. Nutrient agar plate culture is efficient for diagnosis of strongylidiasis, but it has not been evaluated for diagnosis of trichostrongyliasis. Therefore, this study was undertaken to compare efficacy of agar plate culture and formalin-ether concentration methods in laboratory diagnosis of human trichostrongyliasis. During 2013-2014, a total of 970 fresh stool samples were collected from Giulan, Mazandaran and Khouzestan Provinces, and also from patients referring to Helminthological Laboratory of School of Public Health, Tehran University of Medical sciences. All samples were examined for Trichostrongylus by nutrient agar plate culture and formalin ether concentration methods. Considering parasitological results as golden standard, agar plate culture detected 45 true positive Trichostrongylus infected cases. The sensitivity of agar plate culture and formalin ether concentration was 88.23% and 62.75%, and specificity were 98.12% and 100%, respectively.Agar plate culture can be used for detection of Trichostrongylus larvae. Its sensitivity is higher than formalin ether method, but its specificity is lower.

Keywords: agar plate, Trichostrongylus, diagnosis, Iran

#### ECTOPARASITIC INFESTATION OF HOUSE MICE IN KERMAN

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Rodents are one of the biggest challenges in public health. Not only they cause significant economic losses but also, they have a major role in transmission and spreading infectious and zoonotic diseases. Moreover, ectoparasites of mice such as ticks, lice and fleas can transfer a wide range of diseases to human. Identification and control of rodents' parasitic diseases is of great importance. In this study, 100 mice were captured by live-trap from different places in Kerman city in the spring and summer of 2014. All of the rodents were identified as Mus musculus, they examined for ectoparasites. The obtained parasites from each mouse were kept in alcohol 70% and glycerin until identification. 44 mice out of 100 were infested with at least one parasite species. The obtained ectoparasites were consisted of Rhipicephalus sanguineus (2%), Rhipicephalus turanicus (3%), Myocoptes musculinus (25%), Ornithonyssus bacoti (26%), Polyplax sp. (1%) also there was some first stage Diptera larvae from a mouse skin (1%). Since ectoparasites can cause illness and transmit diseases by feeding, it is suggested that control programs for rodents' ectoparasites must perform along with rodents control.

Keywords: ectoparasites, house mice, Kerman





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PREVALENC OF TRYPANOSOMA LEWISI IN WILD RODENTS IN BOYERAHMAD CITY, KOH-GILOYEH AND BOYERAHMAD PROVINCE, IN 2014

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Rodents are the most abundant group of mammals with over 2700 species worldwide, which can be reservoirs of various diseases. Different infectious diseases including parasitic diseases can be transmitted from rodents to humans. Such diseases have been evaluated worldwide, while little is known about the rodent transmitted diseases and their importance in Iran. Therefore, this study aimed to evaluate the prevalence of Trypanosoma lewisi in rodents in Boyerahmad in in 2014. A total of 52 wild rodents were captured alive with cage traps from different parts of the Boyerahmad city. Then rodents were anesthetized with ether and blood films were prepared using the blood taken from the heart. Impression smears were prepared from the spleen and liver tissues. The smears were stained with Giemsa stain and evaluated for detection of Trypanosoma under 100x objectives. Most of the trapped rodents were from subfamily of Gerbellinea. Trypanosoma lewisi was detected in of 6 out of 52 (11.5%) of wild rodents.Rate of infection with Trypanosoma lewisi in rodents in Kohgiloyeh and Boyerahmad province was considerable.

Keywords: Trypanosoma lewisi, wild, Rodent, prevalence, Boyerahmad

#### THEILERIA LESTOQUARDI AS A MAIN CAUSA-TIVE AGENT OF OVINE THEILERIOSIS IN NORTH OF IRAN

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Theileria species are common in tropical and subtropical regions including Iran. Little records are available about epidemiological aspects of ovine theileriosis in Mazandaran Province, thus the present study was aimed to determine the prevalence of the Theileria infection and identification of their species in small ruminants in this area. Twenty-two herds in Mazandaran and Golestan provinces, located in North of Iran, were selected for this study. Each of herds had around 100 animals including sheep and goats. Ten animals from each herd, suspected to theileriosis, were randomly selected. The sampling was performed between 2012- 2013 during summer. Consequently 220 thin and thick blood smears were collected from animals. In this study, we used standard and semi-nested PCR methods for the detection and identification of Theileria spp, respectively. From a total of 220 (9%) blood films including 160 from sheep and 60 from goats, 20 smears indicated Theileria infection in the microscopic examination. The result of the standard 18s rRNA PCR showed 13.6% (n=30) positive for Theileria genus and two cases were indicated mixed infection with Babesia spp. Ten negative microscopic cases were positive using molecular method. The prevalence rate of Theileria infection was 12% (12 of 100) and 15% (18 of 120) in Mazandaran and Golestan provinces, respectively. Moreover, the species of Theileria in all positive cases were found Theileria lestoquardi by semi-nested PCR.Our results showed that ovine theileriosis is enzootic in north of Iran and also Theileria lestoquardi is probably main species of Theileria spp in the area. Our data provide valuable information regarding the epidemiology of theileriosis in sheep and goat in northern regions of Iran which will likely be very favorable for organization and control plans of this disease.

Keywords: ovine theileriosis, *T. lestoquardi*, prevalence, Semi-Nested PCR





Proceedings of 2nd International and 9th National Congress of Parasitology and Parasitic Diseases of Iran (NICOPA 9)

# Guilan, Iran; 20-22 May 2015 ARTHROPODS AND THEIR RELATED MEDICAL HUMAN IMPORTANCE IN THE HOLEY QURAN FECTION

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The Quran, the great and eternal miracle of the prophet Mohammad (pbuh) is the charter of the well being and human conduct. This book has been accountable for the entire requirements of society in all eras and times. As the largest phylum of Animalia kingdom, arthropods have considerable economic, health and ecological impacts. At the first, arthropods' names that directly or indirectly are implied in the Quran was retrieved. Then, for each, the interpretations were given according to the scientific facts and interpret books. Also, their medical importance based on the translation and interpretations were discussed. Nine arthropod names have been mentioned in the Quran.First they have been mentioned as examples and second as divine retribution. Also among arthropod-borne diseases, Plague has been referred to and from the point of treatment application, maggot therapy has also been emphasized.General human life has been programmed in Quran from 1400 years ago. Real and immaculate life will form in the light of the knowledge of the Quran commands and guidelines.

Keywords: Quran, research, arthropods, vector-borne disease

#### HUMAN INTESTINAL MICROSPORIDIAL IN-FECTION IN SOME URBAN AREAS IN JIROFT, KERMAN PROVINCE IN 2013

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Microspordia are single cells eukaryotic obligatory intracellular parasite that are able to produce spore in their hosts. Microsprodial spores can transmit through contact with contaminated water, vegetables and farm animals. Due to appropriate conditions for transmission of the parasites, presence of health problems and lack of information about microsporidial infections, the present study was planned to investigate the prevalence of the microsporidia spores among healthy persons in Jiroft. One hundred stool samples were randomly collected from individuals with unknown immunity status from different areas of Jiroft city in 2013. Conventional formalin-ether method was carried out for all samples. The samples were stained according to Ryan Blue method and then were studied microscopically under 1000X objective. Out of the 100 stool specimens obtained from individuals, microsporidial spores were detected in 29 samples (29%). The highest prevalence, about 31% was found among children up to 9 years old. On the other hand, about 62% and 31% of infected persons had a history of working at the farm and close contact to the domestic animals, respectively. Most of the positive samples were identified among the soft appearance stools, about 24% of individuals identified positive for microsporidia, suffered from gastrointestinal symptoms such as abdominal pain, and bloating. Our results show that microsporidia is one of the most frequent parasites even among healthy individuals particularly the persons with a history of close contact with animals or soil.

Keywords: intestinal microsprodia, prevalence, Jiroft





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#### PREVALENCE OF INTESTINAL PARASITIC IN-FECTIONS IN PATIENTS WITH MULTIPLE SCLEROSIS COMPARED TO THE CONTROL GROUP

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Multiple sclerosis is a demyelinating disease of the central nervous system tissue that is characterized by the triad of inflammation, demyelination, and gliosis. Next to trauma, this disease is considered the second leading cause of neurological disability in the western world, and its increasing prevalence, together with imposing enormous costs of diagnosis, treatment, and rehabilitation on the government, patients, and their families, have heightened the importance of the issue. So far, many studies have been conducted with human and animal models, on the effect of exogenous antigens including bacteria, viruses, and parasites on the person's immune system, incidence and course of the disease, and some are currently being performed. This study calculates and compares prevalence of intestinal parasitic infections in MS patients with a control group, and then considers the risk factors in this disease. A group of 65 patients (53 cases of clinically relapsing-remitting, and 12 secondary progressive cases), and a group of 48 people as the control, from their close relatives were selected. Then level of parasitic infection was determined and compared in these two groups, and in the patients' group their association with age, gender, clinical type and duration of disease was discussed. The findings showed that 78.5% of patients were free of parasites, 3.1% had pathogenic parasites, 18.5% had non-pathogenic parasites and 85.4% of the controls had no parasites, 4.2% had pathogenic parasites, and 10.4% had non-pathogenic parasites.No significant relationship was observed between level of infection in patients and age, gender, clinical type or duration of disease. In addition, no significant relationship was found between risk factors studied and risk factors in MS disease.

Keywords: intestinal parasites, multiple sclerosis, Isfahan, Iran

FREQUENCY OF INTESTINAL PARASITIC IN-FECTIONS AMONG PILGRIMS REFERRED TO CLINICS OF MEDICAL CENTER OF HAJJ PIL-GRIMAGE RED CRESCENT SOCIETY OF THE ISLAMIC REPUBLIC OF IRAN IN IRAQ

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Intestinal parasites of humans are important health problems of most communities. Traveler's diarrhea is the most engaging disorder of pilgrims during the trip. Parasites are one of the etiological agents of diarrhea. This study was carried out with the purpose of achieving a better understanding of the distribution of intestinal parasites. A total of 326 stool specimens were collected and examined by direct examination technique during 2011-2013. The results obtained were analyzed using SPSS software. The general frequency of intestinal parasites was found as 6%. The rates were as following: E. histolytica (4%), Giardia lamblia (2%), Blastocystis hominis (0.3%), and Entamoeba coli (5%). Intestinal parasites are important health problems of pilgrims. In addition to treatment, health education and sanitary measures, control of drinking water, washing hands before meals and after the use of sanitary services would be necessary in controling parasitic diseases in pilgrims.

Keywords: intestinal parasites, diarrhea, Iraq





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PREVALENCE OF STRONGYLOIDES STER-CORALIS AND OTHER INTESTINAL PARASITES IN GERIATRIC CENTERS OF MAZANDARAN PROVINCE, NORTHERN IRAN

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Strongyloidiasis is endemic in Mazandaran province in Iran. The aim of this study was to determine the prevalence of Strongyloides stercoralis and other intestinal parasites in geriatric centers of Mazandaran province. A cross-sectional study was carried out in geriatric centers during 2013-2014. 193 individuals were retaining in the centers that all were examined in this study. Fresh stool samples were collected from all individuals and examined by formalin-ether concentration technique, nutrient agar plate culture, and in case of diarrhea by direct wet preparation, too. Statistical evaluation was performed by SPSS v.16. In general, 15.03% were infected with at least one species of parasite. 13.98% and 1.55% were infected with intestinal helminth and protozoan parasites, respectively. Rate of infection with each species were as followings: S. stercoralis 11.4%, Trichostrongylus spp. 2.6%, Dicrocoelium dendriticum 0.5%, Chilosmastix mesnili 1%, Entamoeba histolytica/dispar 0.5%, and Blastocystis hominis 0.5%. More efforts for increasing sanitation level and prompt diagnosis and treatment of infected persons in these institutions are necessary.

Keywords: Strongyloides stercoralis, intestinal parasites, geriatric, Mazandaran

#### RAILLIETINA (CYCLOPHYLLIDEA: DAVAINE-IDAE) IN PIGEONS OF PIRANSHHAR, NORTH-WEST OF IRAN

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Pigeons of the order Columbiformes are ubiquitous birds and can be found in virtually every town and city around the world. They live side by side with human as a source of food, hobby and experimental purposes. Seeds, fruit, earthworm and insects form the major component of the diets of pigeons may carry infective stages of helminths. Helminth infections can cause mild to severe illness. The current study was designed to provide information on helminth infections of domestic pigeons in Piranshahr, northwest of Iran. Gastrointestinal tract of fifteen pigeons died of uncertain cause were carefully examined for helminths. The parasite isolated of each pigeon transfered into alcohol 70% and identified using available diagnosis keys. Of 15 examined pigeons 7 (46.6%) were infected to parasitic helminths. All of the isolated helminths were of cestodes, Raillietina, (Cyclophyllidea: Davaineidae). Several health problems can affect pigeons, but parasite infections play a major role. One of these important helminthe is Raillietina. The helminth competes for food when it grows to excessive number. In such cases, severe lesions on the intestinal tract will occur. Under heavy infestation, R. echinobothrida listed as one of the most pathogenic tapeworms, causes conspicuous intestinal nodules, with characteristic of hyperplastic enteritis associated with the formation of granuloma. Information on the parasites of domesticated pigeons in this region appears to be poorly documented. Therefore, further comprehensive studies to determine prevalence and distribution of parasitic agents in pigeons of the area is recommended.

Keywords: Raillietina, pigeon, Piranshahr





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RETROSPECTIVE STUDY OF PARASITIC INFEC-TIONS OF GASTROINTESTINAL TRACT IN PA-TIENTS REFERRED TO CLINICAL LABORATO-RIES OF SABZEVAR CITY DURING THE SPRING, SUMMER AND AUTUMN OF 2014

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The prevalence of intestinal parasites is one of the important hygienic parameters to be considered in any society. Monitoring them regularly for proper planning is essential to combat and control of parasitic infections. The purpose of this study was to determine the prevalence rate of intestinal parasites in patients referred to laboratories of Sabzevar city. In this study during the spring, summer and autumn of 2014, a total of 1005 cases referred to 3 large Laboratories of Sabzevar city were evaluated. The results showed that the overall rate of parasitic infection was 4.07 percent. Infection rate was 3.4 % among males and 4.7 % among females. The frequency of intestinal worms (Hymenolepis nana) was 0.09% and intestinal protozoa (Giardia and Entamoeba histolytica and Entamoeba coli) was 3.7 % in which the highest percentage of infection wae allocated to the protozoan parasite Giardia with a frequency of 3.1 %. The prevalence of Entamoeba histolytica and Entamoeba coli was reported 0.3 %. Based on the findings and the higher prevalence of intestinal protozoa, the improvement of the environmental health, personal hygiene and proper diposal of wastes should be continuously emphasized by authoritis.

**Keywords:** parasitic infections, prevalence, frequency, intestinal parasites, Sabzevar INTESTINAL PARASITIC INFECTION INCI-DENCE IN PEOPLE WITH DIABETES AND COM-PARISON WITH CONTROL GROUP IN ISFAHAN, 2014

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Diabetes is one of the most popular metabolic diseases all over the world. This illness may have so many effects on body system because of glucose metabolism disorder. One of the most important disorders is breakdown of immunity system. This breakdown affects both humoral and cellular system, which results in opportunistic infections including parasitic infections. Due to paucity of accurate information about incidence of these infections in diabetic people in Isfahan, the present study was conducted to detect different kinds of parasitic infections in diabetic people and compare them with healthy people. In this comparative descriptive study, 200 people was selected comprised of 100 diabetic people (86 female, 20 male) and 100 healthy people (controls) (80 female, 20 male). This study was done with common laboratory methods from April 2014 till October 2014. At first, people who participated in this study filled out a questionnaire about signs and symptoms and parasitic infections backgrounds. Then, incidence of parasitic infections was analyzed by checking their feces both microscopically and macroscopically. To find all kinds of intestinal parasites, direct and concentration (formalin ether) methods were used, thereafter for further study of intestinal protozoa trichrome staining were used. Acid fast staining for the detection of Cryptosporidium oocyst and other coccidia and modified trichrome webergreen for detection of Microsporidium were used. In this study, from 100 participants in patient group we found 2 cases of Giardia lamblia, 2 Entamoeba coli, 2 Cryptosporidium Sp., 5 Blastocystis hominins, 2 Iodamoeba butschlii, 1 Hymenolepis nana, 4 Endolimax nana, 1 Trichomonas hominis and 2 cases of Microsporidium Sp. In control group, from 100 participants 1 case of Giardia lamblia, 1 case Entamoeba coli, 2 cases o Blastocystis hominis, 1 case of Iodamoeba butschlii, and 2 cases of Endolimax nana were detected. So, in diabetic patients 28 cases and in controls 7 cases were found. There is a significant difference between diabetic and control group in regard to parasitic infections (P < 0.05), which could be related to the weakness of immunity system in diabetic people. With respect to analyses of each kind of parasitic infections in 2 groups, there is a significant difference in incidence of Blastocystis hominis which shows the importance of this infection agent in diabetic people.

Keywords: diabetes, intestinal parasitic, formalin-ether, acidfast, trichrome staining, *Blastocystis hominis* 





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#### KNOWLEDGE, ATTITUDE AND PRACTICE OF THE RESIDENTS OF MEHRAN AND DEHLORAN DISTRICTS ABOUT MYIASIS

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Myiasis is the parasitic infestation of the dead or live tissues in human or animal body by fly larvae (Maggots). Myiasis has a high prevalence in Ilam Province (West of Iran) due to specific climate and extensive livestock farming. Knowing about the knowledge, attitudes and practices of the residents of Mehran and Dehloran districts about Myisis can assist planners for its control. This cross-sectional descriptive study was conducted on 130 participants in Mehran and Dehloran districts in 2014. Data collection was done by a self-report questionnaire that its validity and reliability was confirmed. Data were analyzed using SPSS software. In gender distribution, 78% of the participants were male and 23% female. Overall 44% of them were livestock breeders. The literacy rate was low so that 48% of them were illiterate. The throat, eyes, ears and sometimes sores on the body were attacked by Myiasis flies. Among these individuals, the throat had the highest rate (78%). However, only 60% of participants had referred to physician for treatment and others preferred traditional treatments. The results showed myiasis as a common disease among livestock breeders. Although importance of myiasis is related to many damages to livestock industry, it is zoonotic and humans are affected accidentally, especially those who are in close contact with animals. So, further entomological studies, improving public education especially in livestock breeders and research activities on control and treatment of the disease are suggested.

Keywords: myiasis, knowledge, Mehran, Dehloran, Iran

#### GIARDIA AND CRYPTOSPORIDIUM CONTAMI-NATION OF CARROT JUICE IN AHVAZ AND ITS ASSOCIATION WITH SOME HYGIENIC INDICES

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Foodborne diseases are a common problem in hygiene and public health. In view of the fact that health standards are not met in preparation and distribution of these products, food infections may increase. Given the fact that the root of carrot contacts with soil, it may be infected with several parasites. The significance of Cryptosporidium and Giardia infections as zoonotic parasites on one side, and consumption of carrot juice by a large number of local people on other side encouraged us to perform the present study. In the this study100 samples of carrot juice were collected from five districts in Ahvaz and examined by Ziehl-Neelsen staining for Cryptosporidium(in case of contamination the mean oocysts counted at least in fifty microscopic fields) and by wet preparation method for Giardia cysts. The contamination levels with Cryptosporidium and Giardia were 24% and 7% respectively. Cryptosporidium infection intensity (number of oocysts per ml) was 28 and a maximum was 1985. The contamination levels with Giardia estimated far less than Cryptosporidium which may be due to the lack of effective remedies against Cryptosporidium, existence of a broad host range and robustness of oocysts against antiseptic agents. Accordingly, continuous supervision over the control of disease associated with vegetables and fruit juice and also food production and distribution centers and continuous sampling of products is essential in reducing infection rates.

Keywords: Cryptosporidium, Giardia, juice, Ahvaz





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#### FREQUENCY OF INTESTINAL PARASITIC IN-FECTIONS AMONG PATIENTS REFERRED TO GHAHDERIJAN SINA LABORATORY DURING 2010-2014

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Intestinal parasitic infections are important human health problems of most communities located in tropical and subtropical areas. This study was carried out with the purpose of achieving a better understanding of the distribution of intestinal parasites. A total of 7325 stool specimens were collected and examined by direct examination and formalin-ethyl acetate concentration techniques. The results obtained were analyzed using SPSS software. The general frequency of intestinal parasites was found as 2.8%. The rates were as followings: Giardia lamblia (1.1%), Hymenolepis nana (0.4%), Blastocystis hominis (0.3%), Entamoeba coli (0.5%) and Ascaris (0.2%). There has been a noticeable decline in the rate of intestinal parasitic infections, which may be due to improvements in environmental and personal health, which have occurred through public education campaigns, improved sanitation facilities, proper waste and waste water disposal and control of drinking water.

Keywords: intestinal parasites, Ghahderijan, Isfahan

HEAVY INFESTATION OF PARASITIC CRUSTA-CEAN LERNAEA IN GUPPY (POECILIA RETICU-LATA) IN ORNAMENTAL FISH CULTURE AND PROPAGATION CENTER IN TABRIZ

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Guppy (Poecilia reticulata) is a beautiful and the most popular viviparous freshwater ornamental fish that belongs to the poeciliidae family. These fish are easily reproduced in the aquarium and are well tolerated oxygen levels fluctuations. The purpose of this study was to investigate the cause of heavy losses of guppy in one of the hatchery in Tabriz city. Matherials & Methods:In summer 2012, in an ornamental fish culture and propagtion center in Tabriz symptoms such as lethargy, anorexia with the high mortality were observed in guppy fish. In clinical studies, severe parasitic infestations were observed that were similar to Lernaea sp. About one month before the onset of symptoms, a number of new guppies were added to the hatchery. To determine the cause of mortality, 20 live guppy fish with clinical signs transferred to Ornamental Fish Clinic in Faculty of Veterinary Medicine of University of Tehran. Then wet smear was prepared from skin and fins of foresaid fish and examined under a light microscope. Then, fish were euthanized and necropsied. Macroscopic parasites were isolated from skin and fins by the forceps and were examined under a stereo microscope and light microscope. Internal organs such as intestines were examined for parasitic infections. No parasites were seen in wet smears of the skin and gills of fish. Also, no parasites were seen in examining the internal organs. Microscopic scrutiny leads to definitive diagnosis of severe infection with Lernaea cyprinacea. For the rest of the sick fish, simultaneous treatments were done with trichlorfon and separation of Lernaea by the forceps. After several days, mortality was ended. Infection with Lernaea cyprinacea had a relatively high intensity, and most likely the main factor of the fish problem was involvement with this parasite.

Keywords: Lernaea cyprinacea, guppy, Tabriz





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PARASITIC INFECTION OF STRAY DOGS IN

QAZVIN, GUILAN AND MAZANDARAN PROV-INCES, IRAN

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Stray dogs are in close contact with human societies particularly in rural areas and country sides, so recently, several studies on parasitic infection in carnivores has been done in Iran due to the hygienic and economical importance of controlling the infection as one of the important major issues of public health . Therefore, stray dogs should be considered as reservoirs of common zoonotic diseases and detection and controlling the parasitic infections must be considered by health authorities as one of the enormous economic losses factor. In October 2012 till March 2013, a total of 85 stray dogs were collected alive using bating methods from Qazvin, Guilan and Mazandran provinces. At first, after the tranquilization with 1.5 -2 cc Ketamine, the blood samples were taken for next experiments, then animals were euthanized with over doses of sodium pentobarbital solution. After necropsy and isolation of parasites, specimens collected from different organs were kept in 70% ethanol for more studies. The intestinal contents were kept in 30% Formalin and after one month the intestinal parasites were isolated. In review, 83.5% of 85 stray dogs had at least one species of intestinal parasite. In addation, Dirofilaria immitis (30.58%), Linguatula serrata (20.58%) and Dioctophyma renale (3.52%) were detected. Overall, 16 different parasitic species including 7 Cestoda species, 7 Nematoda species, 1 Acanthocephala and 1 Pentastomida species were detected from total isolated parasites.At least 12 species of isolated parasites in this study were considered as zoonoses, representing the importance of the role of stray dogs as parasitic infection reservoirs. The majority of obtained parasites and also the maximum diversity in parasitic infections were found in dogs collected from Guilan province.

Keywords: parasitic infections, dogs, Iran

#### PROTOZOAL INFECTION OF THEILERIA AN-NULATA AND THEILERIA ORIENTALIS IN GO-LESTAN PROVINCE, IRAN

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Nowadays molecular assays, due to their high sensitivity and specificity rates, are more employed than the traditional methods, e.g. Giemsa staining and serology, for detection of piroplasmosis in domestic animals. Therefore, in order to diagnose Theileria annulata and Theileria orientalis in traditional cattle breeding in two ecological regions of Golestan province, north of Iran, we examined the blood samples of 160 cattle during 2009 to 2010. DNA was extracted from all blood samples. Tbs-S/Tbs-A primer set was used to amplify the both Genus of Theileria and Babesia species in PCR step. For detection and differentiation of Theileria annulata from Theileria orientalis, we examined all positive cases by Semi-Nested PCR method, using specific primers. The results revealed 10% of native cattles in dry areas as well as 5% of native cattle in wet areas harbored Theileria annulata, whereas the infection rate of Theileria orientalis in dry areas was 8.75% and in wet areas was 2.5%. This is the first report from Iran indicating the presence of Theileria orientalis and the mixed infection of both Theileria in native cattle of the country, confirmed by molecular procedures.

Keywords: Theileria annulata, Theileria orientalis, Golestan





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#### PREVALENCE OF INTESTINAL PARASITES IN PATIENTS OF REHABILITATION CENTERS, RASHT, 2012-2013

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Parasitic disorders affect the quality of life worldwide, especially in developing countries. Intestinal parasites can cause a variety of signs and symptoms in infected patients. In institutional environments, rehabilitation centers and crowded places people are in close contact with each other and the sanitation standards is low and the environment is suitable for development and exchange of these parasites. The purpose of this study was to investigate the prevalence of intestinal parasites among people living in rehabilitation centers in Rasht during 2012-2013. A descriptive cross-sectional study was performed by cluster sampling method on 350 individuals residing in 25 rehabilitation centers in Rasht. Factors associated with parasitic infections and demographic information was recorded in a questioaire. One stool sample was collected from each individual. The samples were studied using formalin-ether and agar plate methods. Data were evaluated by SPSS ver.18 software using descriptive statistics and T-test and Chi-Square test. In 60 (17.1%) persons one or more parasites were seen; Blastocystis hominis infection in 31 cases (9.8%), Entamoeba coli in 14 (4%), Giardia in 10 cases (9.2%), Endolimax nana in 2 cases (0.6%), and Strongyloides stercoralis larvae in 6 (1.7%). Also co-infection with two or three parasites was detected. There was significant relation between sex and type of center with intestinal parasitic infections. The present study showed that intestinal parasites have a relatively high prevalence in studied population. Because of dangerous complications due to intestinal parasites, preventing measures, educational programs for the staff, regular stool examination for individuals living in rehabilitation centers and treatment of infected persons can reduce adverse effects and complications of parasitic diseases in studied population.

**Keywords:** intestinal parasites, rehabilitation centers, prevalence, Guilan

INVESTIGATION ON INFECTION TO EXTER-NAL PARASITE IN STRIPED CATFISH, PAN-GASIANODON HYPOPHTHALMUS (SAUVAGE, 1878) & CARNIVORE GIANT PANGASIUS, PAN-GASIUS SANITWONGSEI (SMITH, 1931)

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Pangas is native to the East and South-eastern Asia and is a member of the Order Siliformes and family Pangasiidae that contains two different species: one species is carnivorous, extremely aggressive, black in color with sharp dorsal fin and the other is vegetarian and peaceful and have silver body with horizontal grey line and also is seen white and albino. Striped catfish have very low sight and almost finds food with smell and sensor of barbells and for this reason it is extremely fearful and then is not suitable for keeping in an aquarium. Because of its strong sight and comfort, only carnivorous pangas is suitable for keeping with carnivorous fishes. In order to more improving knowledge about infectious pathogens of this species specially parasitic infections, 50 sample were selected randomly from 10 aquarium stores in Tehran and Isfahan and studied at aquatic animal disease laboratory in the College of Veterinary Medicine, University of Tehran.After checking the appearance of skin, fins and gills, wet mounts were prepared and examined using light microscopy in different magnifications. Results; in striped catfish, infestation relative to Ichthyophthirius multifiliis, Trichodina and Ancylodiscoides were observed. Infestation with Ichthyophthirius multifiliis was 25%, Trichodina 33%, and monogen Ancylodiscoides parasites was 16.5%. Number of Ichthyophthirius was 1-50, Trichodina was 1-50 and Ancylodiscoides was 13 in ×40 magnification. In carnivorous Giant pangasius, Infestation relative to Ichthyophthirius multifiliis was 26% and Ancylodiscoides 2.5%. Number of I. multifiliis was 1-20 in ×40 magnification. Results show that infestation rate with Ichthyophthirius multifiliis in this species was high, similar to that of other freshwater fish species.

**Keywords:** pangas, parasite infections, *Ichthyophthirius multifiliis*, *Trichodina*, *Ancylodiscoides* 





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THE INVESTIGATION OF INFECTION WITH EXTERNAL CRUSTACEAN AND STALKED CILI-ATED PARASITE IN ORNAMENTAL KOI FISH (CYPRINUS CARPIO) IN ORMENTAL FISH CEN-TERS OF TEHRAN

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Ornamental koi fish (cyprinus carpio), from cyprinidae family, is the most beautiful and most popular aquarium fish that lives in fresh water. In addition to its beauty, this fish is one of the most resistant species of cyprinidae. However due to the poor managemet of propagation and culture centers throughout the country, it is subejected to various contaminations, especially parasites infestation. This study focuses on studying crustacean parasite and stalked ciliate external parasite.In fall 2014, 80 pieces of healthy-looking ornamental koi fish were randomly collected from the culture centers all around Tehran and the alive fish were transferred to the labratory of aquatic animal diseases of veterinary faculty, university of Tehran, in order to detect the probable external parasites. At first the smples were investigated macroscopically, then, wet smears were prepared from the skin and fin, and studied microscopically. In the current investigation, it was found that almost 60.1% of the fish were infected with Lernea (Lernea cyprinacea) and 1% was infected with Argulus (Argulus foliaceus) and almost 5.1% was infected with Epistylis sp.For curing infected fish with stalked ciliated, a short-term salt bath was used and beacuse of the low number of Argulus, they were removed by forceps. In the case of Lernea, two kinds of treatment were performed: applying trichlrophen poison and removal using forceps. After one month, these fish were examined again and no sign of infection was observed.koi fish are kept in earthen ponds and these pools are filled with river water, therefore this high infection with Lernea may be due to the probable entrance of parasites into these culture centers through the river water.

Keywords: koi fish, Lernea, Aargulus, stalked ciliated

# FREQUENCY OF THEILERIOSIS AND ANAPLASMOSIS IN REFERRED LIVE-STOCK TO VETERINARY CLINICS IN ZABOL, SOUTHEAST OF IRAN

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Theileriosis and anaplasmosis vectored by ticks are important rikettsial and parasitic diseases. These diseases can bring economic losses in livestock industry. Despite numerous studies and wide distribution of the diseases in Iran, prevalence of diseases in the southeast of Iran especially Zabol district is unclear. Therefore, this study aimed to assess the prevalence of Theileria and Anaplasma in the animals referred to veterinary clinics in Zabol district in 2014. In this cross-sectional descriptive study, data were recorded for each animal including sex and age in an especial questionnaire. 472 Marginal ear vein blood samples were taken to detect theileriosis in 280 sheep, 101 goats and 91 cattle and to detect anaplasmosis in 260 sheep, 99 goats and 91 cattle. Thin and thick Blood smears were prepared. Blood smears were fixed with methanol, stained by Giemsa and then examined for the presence of Theileria and Anaplasma recketsial bodies under a light microscope. The obtained data entered in a SPSS software 20.0 analyzed by chi-square test (P<0.05 was considered significant). The highest rate of infection was observed in cattle under one year. Significant difference between sex and Theileria infection in cattle was not observed. No significant differences were found between the age and sex of animals with Theileria infection in sheep and goats. Also, the results showed that 3 sheep (1.2%), 4 goats (3.92%) and 11 cattle (11.22%) were infected with Anaplasma. No significant differences were found between the age and sex of animals with Anaplasma infection. These finding showed a high frequency of theileriosis especially in sheep and also presence of livestock infected with Anaplasma in this area. Although the prevalence of anaplasmosis in this region was not high; higher prevalence of this disease is expected because of the presence and aggregation of common vectors for both Anaplasma and Theileria. Therefore, sudden outbreak of these diseases can be prevented by vector control programs in Zabol district.

Keywords: theileriosis, anaplasmosis, frequency, livestock, Zabol





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SURVEY OF NEOSPORA CANINUM CONTAMI-NATION IN CATTLE IN QAZVIN PROVINCE US-ING ELISA AND PCR

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Neospora caninum is an obligate intracellular protozoan of the phylum apicomplexa, which causes abortion and great economic losses in cattle worldwild. Cattle is the intermediate host and dog can be infected as definitive host. Among animals, the infection in cattle is of great importace. Horizontal transmission of disease happens through drinking water and foods contaminated with parasite oocys, and the vertical transmission through the placenta to the fetus. Given the importance of this disease in cattle abortion and presence of some reports of Neospora caninum infection in the country, the present study was planned and performed. The purpose of this study was to determine the prevalence of Neospora caninum in cattle using ELISA and PCR in the province of Qazvin. 50 plasma samples of cows were studied for the presence of 328bp fragments of the NC5 gene using primers of Neospora Np21+ caninum, (5'-CCCAGTGCGTCCAATCCTGTAAC-3') and Np6+(5'-CTCGCCAGTCAACCTACGTCTTCT-3'), by PCR and also for the presence of anti parasitic antibodies by ELISA. In this study, 14 samples simultaneously had Neospora caninum DNA and were antibody positive (28%) and the rest were negative for both.Similar seroprevalence studies performed by Iranian scholars have indicated the presence of the parasite in other parts of the country. It appears that Neospora caninum is also prvelent in Qazvin province. Neospora caninum DNA can present in blood samples of all seropositive cows which showing the possibility of vertical transmission of parasite.

Keywords: Neospora caninum, PCR, ELISA, Qazvin

#### IDENTIFICATION OF DICTYOCAULUS SPP. LARVAE IN A SHEEP COPROLITE FROM THE ARCHEOLOGICAL SALT MINE OF CHEHRABAD, NORTHWESTERN IRAN

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Paleoparasitology, the study of human and animal parasites in biological remains, is obtained from archaeological sites. The aim of this study was to diagnose the intestinal parasites of the sheep taking advantage of the coprolite obtained from the Chehrabad archaeological site, Zanjan, Iran. The preservation condition of the organic remains in this archaeological site is comparable with other situations elsewhere. Material & Method:Rehydration of the sample was performed using TSP solution. Several drops of formalin were added to the solution in present work in order to avoid fungal growth. After 10 days, the entire sample was carefully examined. To start paleoparasitology analysis, dating of the obtained materials has been already carried out in archeology section. Result: Out of the 153 prepared slides, larvae of Dictyocaulus spp. were observed in 2 slides. In this study the appropriate preservation conditions of salt on this nematode larvae were clearly seen. Identification of this parasite in only one feces of sheep (0.3gr) could be attributed to a possible high prevalence rate of sheep pneumonia due to Dictyocaulus spp in Sassanid era (500 CE). This report is the oldest evidence of the presence of this parasite in Iran.

Keywords: Paleoparasitology, Iran, Dictyocaulus





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AN ANALYSIS OF EFFICACY OF PROTEOMICS METHOD IN MEDICAL MANAGEMENT-REVIEW ARTICLE

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Proteomics is relatively new method in study of proteins, specially their structures and functions. The term proteomics comes first in 1997 to make an analogy with genomics, the study of the genome. The methods of proteomics are widely used for some microbiological researches and for predicting some types of suspicious diseases even for special gene expression. Materials & Method: Medline, PubMed, Scopus and Cochrane were searched up to 10 December 2014 using "Proteomics Method" and "Proteomics Technology" as keywords. All the associated articles were assessed, and the eligible articles were reviewed. Finally 104 relevant papers were found. Result: A study in Iran in 2014 revealed that we can diagnose some kinds of kidney diseases by finding some biomarkers (like fetuin-A) in urine rather than biopsy of kidney. In another survey in Alzheimer's disease, elevations in beta secretes create amyloid/betaprotein, which causes plaque to build up in the patient's brain, which is thought to play a role in dementia. By proteomics we can target this enzyme to reduce the amyloid/beta-protein and so decreasing the progression of the disease. The protein composition of sputum most faithfully reflects the state of the lungs. Sputum samples were collected from 65 patients with active pulmonary tuberculosis and 38 healthy controls. Comprehensive proteomic approaches were used to profile the proteome changes of host sputum in response to Mycobacterium tuberculosis infection using two dimensional electrophoresis. Metastases are responsible for most of the cases of death in patients with solid tumors. Up to now, proteomics has enabled the identification of number of metastasis-associated proteins and potential biomarkers in cancer tissues, microdissected cells, model systems, and secretomes. Another research done recently shows that heart disease is commonly assessed using several key protein based biomarkers. Standard protein biomarkers for cardiovascular diseases include interleukin-6, interleukin-8, serum amyloid a protein, fibrinogen, and troponins. Cardiac troponin I (cTnI) increases in concentration within 3 to 12 hours of initial cardiac injury and remains elevated days after an acute myocardial infarction.Proteomic technologies will play an important role in drug discovery, diagnostics and molecular medicine because it is the link between genes, proteins and disease. Despite the power of proteomic techniques several limitations remain both in theory and in practice, for instance, in humans, proteins have a dynamic range of expression of more than six orders of magnitude rendering simultaneous analysis of all system components extremely difficult.

**Keywords:** proteomics, proteomics technology

THE EFFECTS OF OSTERTAGIA OCCIDENTALIS SOMATIC ANTIGENS ON OVINE TLR2 AND TLR4 EXPRESSION

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Recognition of helminth derived pathogen associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs), including toll like receptors (TLRs) is the first step towards initiating anti-helminth immune responses. Using somatic antigens of Ostertagia occidentalis, an important abomasal parasite of ruminants, the expression of ovine TLR2 and TLR4 in peripheral blood mononuclear cells (PBMCs) was analysed by real-time quatitative reverse-transcription polymerase chain reaction (qRT-PCR). Somatic antigens of O. occidentalis were prepared to stimulate ovine PBMCs in a time and dose dependent manner. Result: A high expression of TLR2 and TLR4 was observed in PBMCs cultured with somatic antigens of the parasites specially when PBMCs were cultured with 100 µg/ml of somatic antigens and incubated for 2h. Up-regulation of TLR2 expression was more pronounced and evident in our study. The results presented in this study suggest that, somatic antigens of O. occidentalis have immunostimulatory and dominant role on peripheral immune cells. This study provides, for the first time, evidence of induction of TLRs in ovine PBMCs by somatic antigen of O. occidentalis.

Keywords: Ostertagia occidentalis, peripheral blood mononuclear cells, toll-like receptors





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#### LIMITATIONS OF LARVAL CULTURE AND AL-TERNATIVE DNA-BASED METHODS FOR DIAG-NOSIS OF GASTROINTESTINAL NEMATODE IN-FECTIONS IN LIVESTOCK

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The specific diagnosis of gastrointestinal nematode infections in ruminants is routinely based on larval culture technique and on the morphological identification of developed thirdstage larvae. However, research on the ecology and developmental requirements of different species suggests that environmental conditions (e.g., temperature and humidity) for optimal development to occur vary between the different species. Thus, employing a common culture protocol for all species will favor the development of certain species over others and can cause a biased result in particular when species proportions in a mixed infection are to be determined. For the identification of different genera present in mixed nematode infections, fecal culture methods have been developed to hatch eggs and identify first-stage (L1) or infective L3s. A number of different protocols have been described varying in temperatures, times and media used for larval culture. Incubation temperatures ranging from 21 to 27°C have been recommended and probably, the most widely employed protocol in veterinary laboratories suggests incubation at 27°C for 7 days. Culture conditions (27°C for 7 days) are suitable for most species infecting small ruminants, but free living stages of Teladorsagia (Ostertagia) species develop better at somewhat lower temperatures. Also developmental success in fecal cultures was lower for Te.circumcincta than for T. colubriformis when cultured alone or concurrently. Significant progress in this field has been made leading to the adaptation of PCR and RT-PCR technology for the specific identification of livestock nematodes in a number of veterinary diagnostic laboratories and research groups around the world. The routine application of PCR enables better diagnosis i.e. faster, more sensitive and specific results and consequently, a better monitoring and understanding of the prevalence and distribution of livestock nematodes despite its own limitations, requiring the presence of eggs in feces. Currently, the technology has not replaced microscopy but should be used in conjunction with information on egg counts and/or other alternative tests, history and clinical signs of disease. In short, PCR/RT-PCR based approaches do overcome major limitations of larval culture.

Keywords: larval culture, diagnosis, PCR, nematode infection

#### IN VITRO EFFECTS OF MUSA X PARADISIACA EXTRACTS ON FOUR DEVELOPMENTAL

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Gastrointestinal nematodes (GIN), especially Haemonchus contortus, had been classified as a major health problem for small ruminants. Musa x paradisiaca is a plant of medicinal interest in human medicine. The leaf and stem are used to treat diarrhea; the stem is good for asthenia and wounds, and the leaf for the treatment of inflammation, headache and rheumatism. This study was carried out to evaluate the in vitro effect of Musa x paradisiaca stem and leaf against the parasitic nematode of small ruminants namely Haemonchus contortus. Three extracts (aqueous, methanolic and/or dichloromethane) of Musa x paradisiaca stem and leaf were tested in vitro on four developmental stages of H. contortus using egg hatch assay (EHA), larval development assay (LDA), L3 migration inhibition assay (LMI) and adult worm motility assay (AWM). The highly significant (P < 0.0001) ability to stop larval development (inhibition >67% for each extract) and the negative effect of the dichloromethane extract of leaf on adult worm motility (43% of inhibition of motility after 24 h of incubation) compared to the negative controls, suggest anthelmintic properties of Musa x paradisiaca stem and leaf against H. contortus. The active principles responsible for the activity could be secondary metabolites such as terpenoid and flavonoid compounds present in the leaf and stem of the plant. The results indicate that Musa x paradisiaca has some in vitro anthelmintic property on H. contortus. The two organs stem and leaf- influenced the larval development of H. contortus. The methanolic extract of the two organs was the most efficacious.

**Keywords:** *Musa x paradisiacal*, *Haemonchus contortus*, in vitro effects





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PREVALENCE OF TOXOCARA CANIS INFESTA-TION IN OWNED DOGS REFERRED TO THE SMALL ANIMAL CLINIC IN THE CITY OF AMOL

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Toxocariasis is a rare parasitic infection caused by the roundworm Toxocara canis. Larvae of the parasite are capable of transmiting to humans through the feces of infected animals. Infection is especially very common in children because they are usually in contact with parasite ova when playing with soil in sandboxes and parks. Although most people who are infected with the larvae of the parasite do not show any symptoms, symptoms such as cough, headache, fever and stomach pain can be observed in some circumstances. In rare cases, larvae of parasite infect organs such as liver, lungs, eyes or brain and cause severe symptoms such as fatigue, loss of appetite, difficulty in breathing, blurred or cloudy vision. Given the importance of the subject, evaluation of the parasite in owned dogs is considered and this research has been done in this direction. Material & Method: For this purpose, the stool samples of 100 owned dogs were collected at small animal clinic in Amol in 2013. It should be noted that the investigated dogs had no digestive problems such as diarrhea, abdominal pain, etc. In the laboratory, stool samples were examined by the Telman method. The results of laboratory examination showed that in 28% of cases, stools were positive for Toxocara canis ova. The results of the present study indicated high prevalence of Toxocara canis in owned dogs of Amol city and potential risk of infection for children while come into contact with apparently healthy dogs.

Keywords: prevalence, *Toxocara canis*, dog, amol city

A CASE OF SEVERE INFESTATION OF MOUN-TAIN GOATS (CAPREOLUS CAPREOLUS) WITH DICTYOCAULUS SP PARASITES IN MAZANDA-RAN (AMOL CITY)

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Dictyocaulus is one of the round worms, harmful for animals in areas with a temperate climate. This worm is responsible for pulmonary bronchitis in domestic and wild ruminants, especially in endemic areas. Young animals, especially in the first grazing season, most likely suffer from infections caused by this parasite. Larvae, stimulates the lungs and airways, which finally will increase mucus secretion. These parasites can lead to congestion and obstruction in the airways. Damaged cells are covered by connective tissue (fibrosis), which reduces the animal's breathing capacity. In addition, secondary infection with bacteria or viruses can also occur. Finally, all of these factors can cause the loss of animals. This article is a case report of severe pollution antelopes with Dictyocaulus parasite in Amol. Material & Method: In the autumn 1392, environmental experts in Amol found a dead antelope's head with 1.5 year old. Then, the carcass of animals was immediately transferred to veterinary network in the city and autopsy was performed on it. Inspection of animal autopsy showed severe pulmonary emphysema. In addition, there were large numbers of nematodes in the pulmonary tract (trachea and bronchi). Subsequently, nematodes were collected with forceps and placed in a solution containing 70% ethanol and 5% glycerol and for the final diagnosis was ported to the laboratory research center of Mazandaran. In macroscopic examination, lung tissue inflammation, emphysema, pulmonary edema and also tracheal secretions and Bronchiole is well observed. In addition, microscopic examination on parasites confirmed the Genus Dictyocaulus.Due to the severity of contamination with Dictyocaulus and pathological effects associated with the parasite, it can be concluded that the death of the animal has been due to severe infection with this nematode.

Keywords: infection, mountain goats, *Dictyocaulus*, Mazandaran, Amol





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PREVALENCE OF HEART WORM OF STRAY DOGS IN QOM IN 2010-2011

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Heart worm of the domestic and wild carnivores, humans and other animals have been globally reported. Adult worm lives in right ventricle, pulmonary artery and posterior vena cava and its microfilaria is found in the peripheral circulation. These parasites can produce human pulmonary dirofilariasis it is important to study this zoonoses. The purpose of this study is to determine filariosis in stray dogs in the city of Qom. Sixty one dogs collected by the council on the destruction of stray dogs in Qom city and dissected in the Necropsy Hall of Faculty of Veterinary, Science & Research Branch, Tehran University. The heart, lungs and pulmonary artery of dissected dogs were examined and the collected worms were sent to laboratory in alcohol glycerol. Of 61 stray dogs, 3 (4.91%) were infected with adult worms of Dirofilaria immitis. Two adult worms were found in pulmonary artries of a male stray dog (%15.38), 3 worms (23.07%) in the right ventricle of a female dog and 8 (61.53%) adult worms in the right ventricle of a male dog.Removal of stray dogs and treatment of sheepdogs is proposed for control of the disease in study area.

Keywords: heart worm, stray dogs, Qom.

MORPHOLOGICAL CHARACTERIZATION AND DETERMINATION COMPLETE SEQUENCE OF ITS2 REGION OF OSTERTAGIA TRIFURCATA IN SHEEP AND GOAT ISOLATE IN IRAN

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The stomach worm, Ostertagia trifurcata, is the main parasite of domestic ruminants especially sheep and goat in Iran. In acute infections this nematode causes gasteritis in animals. The patogenecity of Ostertagia sp. is usually high. Ostertagiasis can lead to weight loss, anemia, reduced milk and wool production, with potentially maldigestion of proteins in alimentary canal. In the present study ITS2-rDNA and morphological characteristics of O. trifurcata from sheep and goat isolates from Iran were evaluated. Sixty of O. trifurcata male adult worms were collected via necropsy of abomasum from sheep and goat from different localities in Iran. Specimens were morphologically identified based on the length and shape of spicules. The ITS2 of ribosomal DNA were amplified by PCR and sequenced from individual nematodes. The mean length of spicules was 222 - 227 micron in O. trifurcata. The spicules were brown in color and fairly thick in the end divided into three branches - at end of the longer branch a thick botton is observed and near it two thin branches are visible. The PCR product showed 946 bp. O. trifurcata ITS2 sequence and showed 92% similarity with Marshallagia marshalli and 97% similarity with O. circumcincta species. Morphological and molecular characterization clearly determine O. trifurcata as prevalent abomasum nematode in sheep and goat of Iran. In other words, the morphological findings are in full compliance with the information in the world while the results of molecular analysis revealed a very close similarity (97%) with sequences corresponding O. circumcincta.

Keywords: Ostertagia trifurcata, ITS2, sequence, Iran.





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#### PREVALENCE OF OESOPHAGUS WORM INFEC-TION (GONGYLONEMA PULCHRUM) OF CAM-ELS SLAUGHTERED IN SLAUGHTERHOUSES AROUND SHIRAZ

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Introduction& Objectives: The parasites of the family Thelazidae has been considered as an important pathogens in animals. Different species of Gongylonema are upper gastrointestinal parasites of domestic ruminants, equine, pigs, wild animals and have been reported rarely in humans. The purpose of this study was to determine the prevalence of this oesophagal nematode in camels slaughtered in the slaughterhouses of Shiraz. Materials and Methods: During a period of one year from autumn 2011 to autumn 2012, esophagus of 100 camels were examined and evaluated. The samples were randomly collected from the city and villages of Vazirabad, Marvdasht, and Lepui and transported to the Parasitology laboratory of Faculty of Veterinary Medicine, Shiraz.After the opening of the esophagus, the visible surface of membranes cleaned and well rinsed with water. The tests and surveys conducted for the isolation of the parasite. The prevalence of the parasite was low (1%) and only one female camel found to be infected. Given the prevalence of these parasites in ruminants, specific information about the pathology of this parasite is not available or the symptoms of infection with this parasite is not specified, but swelling of the mouth and esophagus severe deformation has been reported. Some researchers believe that this nematode causes neoplastic effects in the wall of the esophagus. Stool-eating beetle in some areas could threaten human health. The study showed camel as a host for the parasite in this area. Supplementary studies in other hosts of this parasite are recommended.

Keywords: Gongylonema pulchrum, camel, Shiraz.

SEASONAL PREVALENCE OF GASTROINTESTI-NAL PARASITES IN SHEEP OF JIROFT, KERMAN, IRAN

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Intoduction & Objectives: Helmnthiasis, especially parasitic gastro-enteritis, constitutes to pose a serious health threat and a limitation to the productivity of small ruminants due to the associated morbidity, mortality, cost of treatment and control measures. Parasites of small ruminants result in low productivity due to stunted growth, poor weight gain and poor feed utilization. The prevalence of gastrointestinal helminths is related to the agro-climatic conditions such as quantity and quality of pasture, temperature, humidity and grazing behavior of the host. For example Haemonchus contortus, found in the abomasum of sheep and goats, causes blood loss resulting in decrease in erythrocytes, lymphocytes, hemoglobin, packed cell volume, body weight and wool growth .The aim of the present study was to investigate the parasite species that mostly occurred in the city of Jiroft to protect the sheep from gastrointestinal parasitic diseases in this city. Gastrointestinal tracts of 154 slaughtered sheep were randomly collected for parasitological investigation, and then different parts of the tract were surveyed. The gastrointestinal (GI) tracts were separated anatomically, then each organ was opened separately and its contents and mucosa were washed in water to remove all parasites. Direct microscopic examination, centrifugation flotation technique and sedimentation technique were used. The study showed that from154 investigated sheep, 35% were found to be infected with one or more parasite species .The prevalence of Parabronema skrjabinema (11.5%) Trichuris globulosa (11%) Haemanchus contortus (8%) were the highest and Oesophagostomom colubianum (4.5%) Marshalagia marshali (3%), Camelostrongylus mentalatus (2.5%), Stilesia globipunctata (2%), Moniezia xpansa (2%), Bunostomum trigonicephalum (0.5%) were the lowest. There was not a significant difference in prevalence of parasites with respect to season (p>0.05) .The prevalence was highest in summer with 50% and were lowest with 38%, 37%, 28% in spring, autmn, winter, respectively. The contamination intensity was low, and this low intensity can't result in clinical symptoms in the animals. Low rainfall in the province and lack of optimum environmental conditions for larval growth can considered among the reasons for this low contamination intensity in the studied animals.

Keywords: prevalence, sheep, endoparasites





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PREVALENCE OF TRICHOSTRONGYLOSIS IN PEOPLE REFERRED TO CLINICAL DIAGNOSTIC LABORATORIES IN MAZANDARAN PROVINCE DURING 2011-2012

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Trichostrongylosis is a common worm infection in humans and animals with an increasing number of cases is being reported from different parts of Mazandaran province in recent years. Based on the importance of this parasitic infection, the aim of this study was to determine the prevalence of Trichostrongylosis infection in people referred to clinical diagnostic laboratories in the province during 2011-2012. A crosssectional study was carried out on 17,328 fecal samples of patients referred to clinical diagnostic laboratories in the cities of the three parts of Mazandaran province in 2011-2012. Fecal samples were collected and examined by direct parasitological method and formalin - ether, using light microscopy for larvae and eggs. Demographic data including age, gender, location, and percentage of eosinophil were recorded and analyzed with chi-square test and T-Test. Of 17,328 individuals, 375 (2.16%) were infected with intestinal protozoan and helminthic parasites of which 102 (26.75%) were infected with Trichostrongylus. The overall prevalence of this parasite among studied population was 0.59%. The highest prevalence of infection with Trichostrongylus was found in ages over 50 years, 47% in male and 53% in female individuals (P< 0.05). The prevalence of infection in rural areas was 58.8%and in urban areas 41.2%. In infected people, 59% had 5-8% eosinophilia and 5% 12-9% eosinophilia. Significant differences was not observed among three study area of the province (p> 0.05).Further studies on diagnosis, treatment, prevention, identification of causative species and geographical distribution of trichostrongylosis in Mazandaran province is recommended.

Keywords: Trichostrongylus, diagnostic laboratories, Mazandaran

#### DETECTION OF PACHYSENTIS CANICOLA (ACANTHOCEPHALA: OLIGACANTHORHYN-CHIDA) IN IRANIAN GOLDEN JACKAL, SISTAN, IRAN

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*Pachysentis canicola* (Acanthocephala: Oligacanthorhynchida) is living in small intestine of wild canids but is rarely observed. The presence of this parasite has already been reported from the Fox in Persian Gulf coastal area of Iran, but this is the first report of the parasite in Iranian golden jackal (*Canis aureus*). In October 2014 during the necropsy of a road killed golden jackal (Zabol district, Sistan, Iran), 11 adult acanthocephalan worms were found in the middle of small intestine. The worms relaxed and kept in ethanol 70% and Glycerin 5%. The acetocarmine staining method was applied and the final identification was done based on morphology of proboscis and body diameters. All of the parasitic specimens were identified as *Pachysentis canicola*. The present case seems to be the first natural infection of *Pachysentis canicola* in *Canis aureus* from Iran.

Keywords: Pachysentis canicola, Acanthocephala, Golden jackal, Canis aureus, Sistan, Iran





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PREVALENCE OF GASTROINTESTINAL TRI-CHOSTRONGYLID NEMATODES IN SHEEP AND GOAT IN ISFAHAN PROVINCE, IRAN

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The most important genera of trichostrongylid nematodes that live in the digestive tract of ruminants include Teladorsagia, Haemonchus, Trichostrongylus, Cooperia, and Marshallagia. Out of the above-mentioned genera, four infect humans including Teladorsagia, Haemonchus, Trichostrongylus, Marshallagia. This study was performed to determine the latest status of zoonotic gastrointestinal trichostrongylid nematodes in sheep and goat, in Isfahan province, center of Iran. The intestines and the abomasums of 109 small domestic ruminants (56 sheep and 53 goats) slaughtered at Isfahan slaughterhouses were collected and examined. Initial separation of nematodes was done via stereoscope. The collected nematodes were stained by lactophenol and azocarmine as a temporary mount. Finally, their genus and species were identified according to morphological characteristics using valid taxonomic keys. Seven species of trichostrongylid nematodes belonging to four genera were isolated from the intestines and the abomasums of sheep and goats. The found species were Trichostrongylus vitrinus, T. probulurus, T. colubriformis, Teladorsagia occidentalis, Teladorsagia circumcincta, Marshallagia marshalli, and Haemonchus contortus. The dominant species in the intestines of sheep and goats was T. vitrinus with frequency of 26.8% and 18.9%, respectively. In the abomasums of sheep, the dominant species was M. marshalli with frequency of 57.1%, and in the abomasums of goats, it was Teladorsagia circumcincta with frequency of 47.2%.Because of zoonotic nature of some species of the parasites, namely T. vitrinus, T. colubriformis, T. probulurus, M. marshalli and H. contortus, measures must be taken to protect the health of people, especially who are directly engaged with livestock.

Keywords: zoonoses, trichostrongylidae, sheep, goat, Isfahan, Iran

#### SEROEPIDEMILOGICAL STUDY OF STRONGY-LOIDIASIS AMONG CANCER PATIENTS UN-DERGOING TOXIC CHEMOTHERAPY AND DI-ALYSIS

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Strongyloidiasis is a chronic intestinal nematode infection, which is especially endemic throughout tropical and warm temperate regions of the world. This infection normally develops chronic, asymptomatic or mild clinical symptoms in immunocompetent individuals, but may be life threatening in immunocompromised individuals such as patients receiving chemotherapy and dialysis patients. Determine the prevalence and distribution of this disease in any area for successful management, and treatment especially the hyperinfection in immunocompromised persons is a key issue. 130 patients who received the cancer chemotherapy and 84 dialysis patients from Bushehr province (total 214 samples) were included in this cross-sectional study. Blood sample was taken from each patient, after centrifugation, serum samples were stored at - 70 C for later examination. The sera were examined for anti-Strongyloides stercoralis IgG antibody by using a commercial ELISA kit. Samples with OD above 0.2 were considered as positive. The association between selected variables and seropositivity was analyzed by Chi square test. P< 0.05 was considered significant. From 7 patients with IgG positive for S. stercoralis, 6 (85.7%), 5 (71.4%), 5 (71.4%), 2 (28.6%), 3 (42.9%), 4 (57.1%), 2 (28.6%), cases stated that have complain of Abdominal, anorexia, diarrhea, pulmonary symptom, itching, emaciation and nausea, respectively, 1 (14.3%) and 5 (71.4%) cases stated that don't disinfect the vegetables and also don't keep pet at home respectively. None of the investigated risk factors showed significant association with serum positivity against S. stercoralis. Risk of S. stercoralis hyperinfection in cancer patients under chemotherapy, dialysis and other immundeficients indivisuals can lead to serious consequences. No significant association was found between Strongyloides seropositivity and different above mentioned clinical sign and symptoms and demograghic characters such as: sex, residence and vegetables consumption. It is necessary to detect the active Strongyloides infectionespecially hyperinfection in cancer patients under chemotherapy and dialysis patients, before any intervention, in order to immediate management and treatment of S. stercoralis infection.

**Keywords:** strongyloidiasis, seroepidemiology, chemotherapy, cancer patients,





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#### HUMAN GASTROINTESTINAL NEMATODIASIS: FETAL COMPLAINT OF CORTICOSTEROID THERAPY

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The aim of study was to highlight the manifestations that appear in immunodeficiency patients administrating corticosteroid therapy when hidden gastrointestinal nematodiasis were present as well. Strongyloidiasis is a parasitic disease created by a nematode called Strongyloides stercoralis. It is known as frequent misdiagnosed nematode and the most forgotten tropical disease in the world due to its nature in human. Material & Method: In a cross sectional study, 50 patients with unknown or suspected causes of death were introduced to mortality committee of a non-private hospital in the first six month of 2014 in Shiraz, Iran. The pesent case was selected and various information and data were studied. Patient is a 54-year old Iranian man formerly confirmed for Pemphigous vulgaris admitted to emergency room of a general Hospital in Shiraz on Aug 2, 2014. Regarding his different finding, primary diagnosis suggested for him at arrival was Pancreatitis, Adrenal insufficiency and Lung cancer but final diagnosiswas registered Diabetes. Histophtolgical biopsy examination on intestine revealed massive strongyloides stercoralis infection in the duodenal mucosa, bowel and gastric wall. Presence of adult females, eggs, and larvae are notable. However, other evidences in his file agreed fetal hyperinfection syndrome due to Strongyloides stercoralis. We emphasize that biopsy is the best method to confirm gastrointestinal strongylidiasis. To start steroid therapy in immunodeficiency patients a prophylaxis with Ivermectin as standard dose should be administered in admission for all immune competent patients especially in suspected and endemic area. Hyper infectious cases should be placed on contact isolation. Hand washing and using of gloves, masks, and aprons are effective methods that could prevent spread to health care workers and other patients. Thus as soon as possible the Hospital Infection Control Committee should be informed to take precautionary activities and mange intersectional coordination.

Keywords: Strongyloides stercoralis, Pemphigous vulgaris, infection, Iran

# TWENTY-TWO-YEARS RETROSPECTIVE STUDY OF THE DISTRIBUTION HUMAN ASCARIASIS AND TRICHURIASIS IN MAZANDARAN PROV-INCE, NORTH OF IRAN

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Ascaris and Trichuris are the most common soil-transmitted worms. The aim of the present study was to investigate the geographical distribution of the two parasites in Mazandaran province as common helminthic parasites from 1981 to 2013. The descriptive study was done by collecting data on the geographical distribution of ascariasis & trichoriasis status in Mazandaran province during 22 years, extracted from review of papers, summaries of the thesis of medical parasitology and internet search engines using software Arc GIS, 9.2 for maping of the geographical distribution of the disease. Based on the maps, the prevalence of Ascaris and Trichuris was divided to five groups. Maximum of the prevalence of Ascaris was reported from rural areas of Tonkabon (16.3%) in 1981-1982. The mean prevalence of Ascaris was observed 2-4% in central and 4% in west of Mazandaran province. The maximum prevalence of the Trichuris in rural areas of Tonkabon was 22.5% in 1981-1982 and the lowest in the cattle breeders in rural areas of province was also 0.06% during 2002-2003. The prevalence of ascariasis & Trichuris in 22 years has shown the pattern of the distribution of two parasitic diseases in this area. Results of this study are beneficial for physicians and clinicians in the diagnosis and treatment of diseases, training centers, researchers and health planners.

Keywords: ascariasis, *Trichuris*, geographical distribution, GIS, Mazandarn





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# SEROEPIDEMIOLOGY OF TOXOCARIASIS IN CHILDREN (5-15 YEARS OLD) REFERRED TO THE PEDIATRIC CLINIC OF IMAM HOSSEIN HOSPITAL IN ISFAHAN

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Human toxocariasis is a zoonotic helmithic disease due to the migration of Toxocara larvae into human organs. Humans, especially children, become infected by ingesting embryonated eggs when come into contact with contaminated soil or eat contaminated vegetables. Three clinical forms of toxocariasis are described including visceral larva migrans (VLM), ocular larva migrans (OLM), and covert toxocariasis. Seroprevalence is high in developed countries, especially in rural areas. Due to less epidemiological information about this disease in Iran and considering the importance of toxocariasis and great impact of the disease on children, the present seroepidemiological study was performed on children attended at Imam Hossein hospital in Isfahan. In this study the sera of children (5 to 15 years old), admitted to pediatric department of Imam Hossein hospital from spring 2013 to spring 2014 were collected. These sera were examined for anti Toxocara antibodies. From 427 children refered to the hospital, 196 children (45.9%) were female and 231children (54.1%) were male. 107 (25.1%) were from rural and 320 (74.9%) were from urban area. 129 (30.2%) had contact with dogs. One child (0.2%) had hypereosinophilia but 33 (7.7%) had eosinophlia, 6 (1.39%) had positive ELISA for T. canis IgG, of them two were male and four were female. Two positive cases were from rural areas and four from urban regions. There was no significant relation between ELISA test results and education of parents, gender, age, place of living and contact with dogs. Our study showed that toxocariasis is present in the children of Isfahan region. So it is recommended that this disease should be considered in the differential diagnosis of children diseases in this region.

Keywords: toxocariasis, visceral larva migrans, seroepidemiology, children.

# PREDICTION OF SECONDARY STRUCTURE OF ITS1, 5.8S, ITS2 GENES IN PARABRONEMA SKRJABINI

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Nematodes are responsible for most of the helminthes diseases of veterinary importance and live in the tissues or organs of every class of vertebrates and even some invertebrates. Parabronema skrjabini is one of the nematodes that affect the abomasum of ruminants. The purpose of this study was to predict the secondary structure of 18S, ITS1, ITS2, 5.8S and 18S genes in Parabronema skrjabini species, which were collected from Sanandaj province (west of Iran) of animal husbandry. DNA extraction was performed. rDNA gene was amplified from each isolate using the primer pairs and was purified. In the present study the RNA secondary structure was predicted using mfold web server by screening for thermodynamically optimal and suboptimal secondary structures (default settings, with  $T = 37^{\circ}C$ ). Energy levels of the presumptive secondary structures were then calculated with Mfold. ITS length was found 607 base pairs (bp). Length of ITS1 and ITS2 were 308 bp and 299 bp, respectively whereas GC% was 38 and 31, respectively. 5.8 S gene length found 158 bp and GC% was 54. The predicted secondary structure for a functional 18S, ITS1, 5.8S, ITS2 and 28S gene, was with bulge, hairpins, helices, interior, external and multi loops form, but we cannot show them here because of abstract limitation.Several authors have emphasized that the secondary, not the primary, structure (i.e. nucleotide sequence itself) of the ITS regions is conserved at higher systematic levels. Ribosomal DNA codes for ribosomal RNA. Ribosomes are assemblies of proteins that are important as drug target. Characters from nuclear ribosomal gene sequences have been used for hypotheses of phylogenetic relationships among even distantly related organisms.

Keywords: secondary structure, Parabronema skrjabini





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MOLECULAR CHARACTERIZATION OF TRI-CHOSTRONGYLUS COLUBRIFORMIS AND T. VITRINUS ISOLATED FROM HUMAN IN GUILAN PROVINCE: THE FIRST REPORT IN IRAN

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Trichostrongyliasis, the infection caused by the different species of the Genus Trichostrongylus, has a worldwide distribution in ruminants. Up to the moment, more the 30 species have been recognized to infect mammals of which 10 species reported from humans. In Iran, 9 species of the genus Trichostrongylus have reported from livestock, mainly from central and southwestern parts of the country. Interestingly, all these species have also been obtained from humans. Close contact with herbivorous animals and use of fresh livestock manure as fertilizer are the main cause of human infections. The fecal materials of a symptomatic patient from Langroud district of Guilan province, who had been diagnosed to be infected with Trichostrongylus spp., were collected after treatment with combination of Mebendazole and Albendazole. The male worms were obtained from the fecal materials identified as T. colubriformis and T. vitrinus by morphological characteristics. For definitive identification of the parasites a Polymerase Chain Reaction (PCR), specific for rDNA ITS2 was performed of NC1: using the primer set 5-ACGTCTGGTTCAGGGTTGTT-3) and NC2: 5- TTAG-TITCTTTTCCTCCGCT-3) (Chilton, 2004). In present study, amplicon of about 328 base pair (bp) was successfully produced by PCR for ITS2 region and using the BLAST system, Trichostrongylus isolates were identified as T. colubriformis and T. vitrinus. Sequences of these two human isolates were deposited in GenBank database (accession numbers: KF826913and KF872228, respectively). This study is the first molecular evidence of human isolates of T. colubriformis and T. vitrinus in Iran. The ITS2 sequence of Iranian T. vitrinus was identical to ITS2 sequences of T. vitrinus from New Zealand (Accession No. KC998731) and United Kingdom (Accession No. AY439027) and that of T. colubriformis was identical to isolates of Iran (animal isolate), Laos, Australia and Malaysia found in GenBank.

**Keywords:** trichostrongyliasis, *Trichostrongylus colubriformis, T. vitrinus*, molecular identification, Guilan, Iran

PREVALENCE OF ENTEROBIUS VERMICULARIS IN 4-7 YEAR OLD PATIENTS REFFERED TO CENTRAL LABORATORY IN EAST AZERBAIJAN PROVINCE, 1392-1393

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Enterobius vermicularis (Oxyuris) is an intestinal nematode with a direct transmission route and a worldwide distribution. Enterobiasis is common in children and has many adverse effects on their health. The noted complications are anorexia, malnutrition, pruritus ani, restlessness and vulvovaginitis. Due to its contagious nature, people may remain infected for years. The specific diagnosis is made by using of scotch tape (Graham method). The purpose of this study was to determine the prevalence of oxyuris in patients (aged 4-7 year old) admitted to the Central Department of Parasitology Laboratory in East Azerbaijan using the tape method from April 2013 to December 2014. Of 1030 examined individuals (54.8% male and 45.2% female), 19 cases (1.84%) were positive. The results revealed that 2.1% of girls and 1.6% of boys were infected with Oxyuris. The most common clinical symptoms in patients was anal itching. Children due to special circumstances are more vulnerable than other groups. Since, personal hygiene and crowding are crucial factors in transmission and distribution of the disease, health education has the most important role in increasing the knowledge of the families and reducing the prevalence of the enterobiasis.

**Keywords:** *Enterobius vermicularis*, prevalence, Graham method, scotch tape





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# PREVALENCE OF STRONGYLOIDES INFECTION IN PATIENT'S REFERRED TO RASHT RAZI LA-BORATORY THROUGH FORMALIN-ETHER AND ELISA

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Strongyloides stercoralis is an intestinal nematode endemic in the tropical and subtropical areas. Parasitological diagnosis based on the fecal examination is usually difficult in cases of chronic and low-level Strongyloides infection. Additionally, it is important to note that a negative result does not necessarily indicate the absence of the infection. This research was performed to study the prevalence of Strongyloides infection in patients referred to Rasht Razi Laboratory using two methods: formalin-ether and ELISA (Enzyme-linked Immunosorbent Assay). This descriptive cross-sectional study was conducted through random selection of 370 patients referred to Rasht Razi Laboratory. The fecal and serum samples were collected. The fecal samples were examined through the formalin-ether method. An ELISA test was performed using an ELISA kit for the diagnosis of human Strongyloidosis (Bordier, Switzerland) and based on the manufacturer's instructions on the serum samples. The prevalence of Strongyloides infection, based on findings in the formalin-ether method, was 1.6%. Regarding the ELISA test, the results proved the prevalence of 15.4%. In the ELISA results, there was a significant relationship between participants' age, residence, level of education and occupation with the prevalence of Strongyloides. However, there was not any relationship with sex. In results of the formalin-ether method, there was only a significant relationship between participants' age, residence and level of education with the prevalence of Strongyloides.Since the ELISA test can determine the chronic and low-level infections with Strogyloides and has higher sensitivity and specificity, it is recommended for epidemiological studies.

Keywords: prevalence, *Strongyloides stercoralis*, ELISA, Rasht, Iran.

# SOIL CONTAMINATION WITH TOXOCARA SPP. EGGS IN PUBLIC PLACES OF ARDABIL CITY

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Toxocariasis is a parasitic zoonosis with worldwild distribution. It is caused by the ascarids of dogs (Toxocara canis) and cats (Toxocara cati). Human become an incidental host for Toxocara when they ingest the eggs of the parasite, chiefly by hand-to-mouth contact, from exposure to areas polluted with Toxocara eggs, such as contaminated soil in public places. Therefore, soil is noted as the main source of transmission of Toxocara infection to human beings. There are no data about soil contamination by Toxocara spp. eggs from Ardabil city. The aim of current study was to estimate the extent of soil contamination with Toxocara eggs in public places. This survey was conducted from March 2013 to March 2014. A total of 200 samples collected randomly from 41 public places in various parts of Ardabil city and examined by microscopy following sodium nitrate flotation. Toxocara eggs were recovered from 14 soil samples indicating an overall frequency rate of 7 percent. This investigation gives baseline knowledge regarding soil contamination with Toxocara spp. eggs in Ardabil city and will provide information for implementation of control measures in the area.

Keywords: Toxocara, soil contamination, public places, Ardabil





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CONTAMINATION OF COMMONLY CONSUMED RAW VEGETABLES WITH SOIL TRANSMITTED HELMINTHES EGGS IN MAZANDARAN PROV-INCE, NORTHERN IRAN

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For a healthy diet, people are usually encouraged to eat many raw (fresh) vegetables and this issue can serve as a risk factor for human infections with soil transmitted helminthes (STH). For this purpose the presence of STH eggs on raw vegetables, including radish, scallion, spinach, lettuce, parsley, green onions and mint from retail markets in Mazandaran province, northern Iran was investigated. A total of 772 fresh vegetable samples which belonged to spring-summer (386) and winterautumn (386) were obtained from retail markets. Each sample was divided into two groups. One group was used as the unwashed sample and the second group was washed with standard washing procedures. Then, samples were examined for helminth eggs by using standard methods. Eggs of STH were detected in 20.2% of unwashed vegetables in springsummer; and 9% of unwashed vegetables in winter-autumn (P < 0.05). No parasites were observed in standard washed samples (P < 0.001). The parasites were detected in unwashed vegetable samples included Ascaris lumbricoides eggs (3.3%), Trichuris trichiura eggs (2.3%), Hook worms eggs (3.1%), Toxocara spp. eggs (2.3%), Trichostrongylus spp. eggs (1.9%), Taeniid spp. eggs (0.9%), and Hymenolepis nana (2.3%).In view of results of this study there is an indication that human infection with soil transmitted helminthes can be acquired through the consumption of unwashed raw vegetables, and standard washing procedure before consumption of fresh vegetables is strongly recomended.

Keywords: soil transmitted helminthes, vegetables, Mazandaran, Iran

# THE REPORT OF HYMENOLEPIS NANA FRA-TERNA INFECTION IN RODENTS OF SANAN-DAJ, WEST OF IRAN

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Hymenolepiasis is the infection caused by two species of the Genus Hymenolepis, H. nana and H. diminuta. The risk of human infection from H. diminuta, known also as rat tapeworm, is very low, because rats are the main hosts of the parasite. The disease is mainly prevalent in tropical areas and is classified among neglected tropical diseases. Although the parasite was isolated from the duodenum of human in Cairo, in 1851 its biology remained unclear for many years. In recent years, with identifying the fraterna strain, the life cycle of H. nana was introduced as an evolutionary process and survival mechanism. Hymenolepis nana fraterna is a race, strain or subspecies of the Genus Hymenolepis, commonly adapted to rodents. It is believed that the human form, H. nana nana, has presumably derived from rodent strain. The rodent form of H. nana is also infective for humans especially in places which rodents live close to human population. 15 rodents including 8 Rattus spp., two Rhombomys and 5 mice were collected from different areas of Sanandaj and transferred to the laboratory. After dissection, intestinal contents were put in saline separately. Parasites were investigated by optical microscope and stained using the Carmine staining. Of the 15 captured rodents, only one Rattus rattus was infected with H. nana. fraterna. Rattus rattus and R. norvegicus are two dominant species of rats living commensally with people in many places of the world. Both species have so adapted with commensal life that are regarded as pests, so the risk of transmission of their parasites to humans should always be considered. Previous studies has confirmed H. nana as one of the most common intestinal parasites of humans in Sanandaj city. This study verifies Rattus rattus as a reservoir host of murine strain (H. nana. fraterna) in Sanandaj and the potential risk for cross-infection of the parasite to local residents.

**Keywords:** *Hymenolepis nana fraterna*, rodents, R*attus rattus*, Sanandaj





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# EVALUATION OF PLASMA GASTRIN CONCEN-TRATION AND MORPHOLOGICAL CHANGES OF ABOMASAL TISSUE IN LAMBS INFECTED EX-PRIMANTALLY WITH MARSHALLAGIA MAR-SHALLI

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Species of Marshallagia are abomasal parasites in free-ranging and domesticated ungulates in temperate climatic zones throughout the world. Pervasiveness of these nematodes is significant in various parts of the world. There has been limited research in the area of Marshallagia marshalli pathogenesis. The aim of this study was to investigate the effects of M. marshalli on the serum gastrin concentration and the morphological changes due to parasitic migration to different parts of abomasal tissue in sheep. Ten lambs, approximately around 6 months old, were divided into two groups of five (A and B). Antihelminthic treatment was done to ensure the absence of any worm contamination in lambs. The sheep from group A were infected intraruminally with a dose of 5000 third stage larvae (L3) of M. marshalli whereas the sheep of group B were not infected. 25000 infected larvae (L3) were cultured from sheep infected with 10000 infective L3 of Marshallagia (donor lamb). Jugular blood samples were collected from each lamb twice a week. Lambs were slaughtered after 30 days and abomasal tissues were sampled for histological examination. Serum gastrin concentrations were measured using human ELIZA kit. Sheep and human gastrin have nearly 80% similarity. So in this study human ELIZA kit was used to measure G17. Since, we have no information of serum G17 gastrin values, the measured values were compared. Based on the results of statistical analyzes no significant differences found between the two groups. The results showed that all infected lambs had mucosal lesions of different levels. Both acute and chronic lesions of abomasal tissue section were seen in microscopic observation. Histologic changes include mucosal cell hyperplasia, loss of parietal cells, and inflammatory cell infiltration, with numerous granulocytes and lymphocytes. Hypergastrinemia has been proposed as a diagnostic marker for abomasal parasitism. In this study, there was no significant difference in lamb gastrin level between control and treatment group. There was massive thickening of the antral mucosa at necropsy and histologically severe inflammation which may have damaged the gastrinsecreting G-cell, resulting in the lower serum gastrin concentrations. The exact effect of M. marshalli infection on the serum gastrin concentration is not known and new researches are recommend to be carried out with more larvae in long time.

Keywords: Marshallagia marshalli, plasma gastrin concentration, abomasal morphological changes

# ENTEROBIUS VERMICULARIS AMONG CHIL-DREN IN RAMSAR CITY IN SUMMER 2014

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Prevalence and epidemiological information regarding intestinal parasites and identification of risk factors in different regions is a prerequisite priority to develop appropriate control strategies. The aim of this study was to determine the prevalence of Enterobius vermicularis and its risk factors in 500 children 2-7 years old in 5 health centers in Ramsar city during summer 2014. In this descriptive study sampling was randomly done from 500 children referred to 5 health centers and the prevalence of Enterobius vermicularis was studied. The questionnaires fulfilled by data collection such as age and gender of child. Scotch test, direct smear test and formalin ether were performed for all samples. The results of the scotch test, direct smear test and formalin ether technique either positive or negative were recorded. Specificity and sensitivity in scotch test compared with direct smear test and formalin ether technique. Result: Out of 500 children, 350 of them (70%) were male and 150(30%) were female. The rate of Enterobius vermicularis in scotch tape test was 5% (25 case) and the prevalence of Enterobius vermicularis in direct smear test and formalin ether was 1% (10 case). So the specify for scotch test in this study was 90%. Our result showed that the prevalence of Enterobius vermicularis is high and parents should be more careful about parasite transmission routs. Therefore, education of parents and children and treatment of infected people are key measures to reduce the rate of infection and transmission.

Keywords: Enterobius vermicularis, children, Ramsar city





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MORPHOLOGICAL AND MOLECULAR DE-SCRIBTION OF TRICHOSTRONGYLUS SPECIES IN MESHKINSHAR SHEEP, ARDABIL PROVINCE, IRAN

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Nematodes of family Trichostrongylidae live in abomasum and beginning of small intestines of ruminants and herbivores. They can be pathogenic in humans as well. In this study, morphological and molecular characterization of Trichostrongylus isolated from sheep in Meshkinshar is investigated. In 2012, the contents of abomasum and small intestine of sheep, slaughtered in a traditional abbotoir in Meshkinshahr were examined and Trichostrongylus nematodes were collected. Morphological characteristics of nematodes were studied after clearing and staining, using a calibrated microscope equipped with a drawing tube. Genomic DNA was extracted and ITS2-rDNA region was amplified by PCR. PCR products were sequenced and compared with sequences in the GenBank database. Three species including Trichostrongylus axei, Trichostrongylus columbiformis and Trichostrongylus vitrinus were identified. All species formed similar band on gel electrophoresis. Analysis of sequences revealed the presence of T. axei (KJ755059.1), T. colubriformis (KJ755060.1) and T. vitrinus (KJ755061.1), all with 100% homology with relevant Sequences in GenBank.Species of T. axei, T. colubriformis and T. vitrinus are comom in sheep in Meshkinshahr, and the dominant species is T. vitrinus.

Keywords: Trichostrongylus, sheep, ITS-2, Iran

# MOLECULAR IDENTIFICATION OF TELADOR-SAGIA CIRCUMCINCTA ISOLATED FROM HU-MAN IN GUILAN PROVINCE, NORTHERN IRAN: THE FIRST GLOBAL REPORT

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Ostertagia and Teladorsagia, also called brown stomach worms, are two genera of gastrointestinal nematodes that infect cattle, sheep and goats and other wild ruminants worldwide, especially in temperate and cool climates. O. ostertagi infects mainly cattle, but also sheep, goats and other domestic and wild ruminants while T. circumcincta(also known as O. circumcincta) is a parasitic nematode that infects sheep and goats worldwide and infects the gastric glands of the stomach and lead to weight loss, decreased wool production and death. Here, the first case of human infection in the world is presented. A 35 year old female residing in Langroud district of Guilan province presented with a three week history of severe and voluminous diarrhea intensified following meals, poor appetite, mild abdominal and epigastric pain radiated to her back, urticaria on the chest and neck associated with itching and rigors which was prominent at nights. Haematological analysis indicated 60% eosinophilia. On stool examination, large numbers of Trichostrongylus ova were seen. She was treated with combination of Mebendazole and Albendazome and her 24-hour fecal material was collected in 70% ethanol. All adult worms were collected and kept in 70% alcohol until molecular and morphological examinations. Genomic DNA was extracted from the samples and second internal transcribed spacers (ITS2) region was PCRamplified and sequenced. The sequences were compared with published sequences in GenBank and phylogenetic analysis was performed using Mega 5.0 software. Two species of the Genus Trichostrongylus (T. colubriformis and T. vitrinus) and a male worm of T. circumcincta were identified based on molecular and morphometric characteristics. Comparison of the second internal transcribed spacers (ITS2) sequence of T. circumcincta isolated in this study with sequences deposited in GenBank showed 0-0.6% intra-species variation. This isolate also showed 100% homology with the reference sequence of T. circumcincta from Iran (Accession nos. JQ989274). The results of the present study verified the possibility of human infections with T. circumcincta, whenever there is a history of eating fresh vegetables, fertilized with fresh livestock manure or close contact with herbivorous animals.

Keywords: molecular identification, *Teladorsagia circumcincta*, *Trichostrongylus*, *Ostertagia*, Iran





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# DIAGNOSIS OF TRICHURIS SPECIES IN SHEEP SLAUGHTERED IN ABATTOIR OF MASHHAD CITY

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Trichuris (whipworms) is a genus of parasite worms from the roundworm family Trichuridae. Trichurias is a soiltransmitted helminthiasis and belongs to the group of neglected tropical diseases, affecting about 604 million people globally. The genus Trichuris includes over 20 species, which infect the large intestine of their host. From August 2013 to May 2014, 275 sheep were analyzed to confirm the presence of gastrointestinal parasitic infection. 128 (46.5%) samples were found positive for Trichuris spp. Totally, 489 Trichuris spp. were found - 63.5% (n=311) female and 36.4% (n=178) male. Worm burden of Trichuris in intestine was 1 to 29. We have used the light microscope for the diagnosis of the species by detecting the spicular sheath. We detected 49% (n=243) T. ovina, 28% (n=135) T. discolor, 17% (n=83) T. Skerjabini, 5% (n=24) T. barbetonensis and 1% (n=4) was unknown. The results show that major nematodes of large intestine belongs to genera Trichuris.sp with high prevalent in the areas of Khorasan. Further studies on the economic importance of helminthiasis and control patterns should be conducted.

Keywords: sheep, Trichuris, prevalence, Khorasan.

# RODENTS PINWORM (OXYURIDAE) INFEC-TIONS IN PARTS OF IRAN DURING THE SUM-MER AND AUTUMN 2014

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Pinworms or oxyurids are intestinal nematodes belonging to the family Oxyuridae. Syphacia obvelata, Asicularis tetraptera and Syphacia muris infect both mice and rats. The later having been diagnosed as the main pinworm of rats. They live in cecum and have been reported from humans but are not major pathogens, although some published work suggests some advers affects on the immune system. According to literature, they are considered as infectious helminthes of human. The current survey aimed to determine the prevalence of common pinworms of rodents in some part of Iran. Total of 100 rodents (43 Mus musculus, 13 Apodemus witherbyi,14 Calamyscus elburzensis, 12 Tatera indica, 8 Microtus socialis, 7 Meriones libycus, 3 Alactaga elater) were captured using live traps during the summer and autumn 2014 from Zanjan, Yasuj, Ahvaz and Bojnord in Iran. After transferring to the laboratory, the rodents and their collected parasites were identified. The results indicated that 33% of examined rodents were infected with oxyuridae nematodes. 495 oxyuridae nematodes collected from 33 infested rodents were related to 2 genera. The rate of infectivity with each species was as followings: Syphacia obvelata 17%, Aspicularis tetraptera 11% and mix infestation were estimated 5%. No rodents were infected with S. muris. All of Meriones libycus, Microtus socialis and Tatera indica were free of pinworms. Parasitic infections of rodents can compromise health of the animal and humans. Among oxyuridae species identified in rodents, some have been reported from human and since rodents are usually infected with a number of zoonotic parasites control of these animals has an important role in safeguarding public health.

Keywords: pinworms, rodents, Iran





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SURVEY OF HELMINTH PARASITIC INFEC-TIONS IN SMALL RUMINANTS OF SHIRAZ, IRAN

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The importance of small ruminants in the supply of food, clothing and livelihood of humans is clear to all and livestock rearing is common in different areas of the world, so addressing the aspects of animals' health and their pathogenic agents is inevitable. Parasitic diseases especially helminth infections cause negative impacts on animal production and significant economical losses to livestock industry. This study aimed to survey helminth parasitic infections in small ruminants in Shiraz, Iran. Rectally, forty samples were randomly taken from sheep and goats of the area. Immediately, the samples were transferred to laboratory of parasitology and floated by Clayton-Lane technique. Prepared slides of each sample were surveyed under microscope for parasites and identified using available diagnostic keys. Overall, 10 (25%) sheep were infection. Isolated parasites and their abundance were as followings: Trichostrongylus spp. (50%), Fasciola (20%), Marshallagia marshalli (20%) and Moniezia benedeni (10%). Although usually mild infections with parasitic helminth have no specific symptoms, in case of severe infection the parasites can be very damaging and deadly. Considering the ability of the helminth in threat of animal health and their negative affects on animal production, surveillance on prevalence of these infections and identification the species of parasites is recommended.

Keywords: parasitic helminth, small ruminant, Iran

DEVELOPMENT OF A SINGLE GLASS MOUNT-ING METHOD USING POLYVINYL ALCOHOL (PVA)-CONTAINING MEDIUM IN COMPARISON WITH CLASSIC METHOD FOR PERMANENT MOUNTING OF SMALL NEMATODES

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Different mounting media and methods are used for permanent mounting of helminthes most of them are accompanied by dehydration. The present study was conducted in order to develop a single glass mounting method using Polyvinyl Alcohol (PVA)-containing medium which has already been used successfully for permanent mounting of heminth eggs ( Sadijadi, 1991) for small nematodes and its comparison with classic method. A total of 594 nematodes including 16 different species from human and animal nematodes were used in this study. 557 Samples were fixed and stained with Formaldehyde Alcohol Azocarmine Lactophenol (FAAL) followed by mounting with PVA containing medium using single coverslip and 37 samples in classic mounting method for comparison. The slides were evaluated in different dates and time over four years. Different photos including normal and phase contrast microscopy were made with different magnification during evaluation time to compare advantages and disadvantages of the methods. The results showed that staining nematodes with FAAL and mounting by use of PVA containing medium using a single glass coverslip, had good results. Slides have high quality after years without substantial degradation and changes in their morphologic structures. In this method, well defined differentiation between different organs of nematodes is comparable with classic method.As, using PVA containing medium for permanent mounting of small nematodes do not need dehydration; it is rapid and provides slides with clear and transparent background comparing to other methods. The advantages of this method such as fast and simple preparation of slides of nematodes with high quality, introduce it as an effective method for permanent mounting of small nematodes. Moreover, the specimens could be transferred into classic method by resolving the medium in water and making new slides with other methods.

**Keywords:** helminths, nematodes, permanent mounting, polyvinyl alcohol, classic method





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DETECTION OF TOXOCARA SPECIES IN NATU-RALLY INFECTED BROILER CHICKENS BY PAR-ASITOLOGICAL METHOD AND PCR-BASED AS-SAY

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Little is known of the molecular detection of Toxocara infection in chickens. A polymerase chain reaction (PCR) method has been used for the differentiation of Toxocara canis and Toxocara cati larvae recovered from tissue and identified from microscopically observations. Thirty three, 35-47 days old broiler chickens used for examination of Toxocara larvae. The duodenum, livers, lungs, hearts, kidneys, and brains of chickens were examined using the routine digestive method and DNA from each tissue extracted as template for PCR assay. The results showed the presence of Toxocara canis larvae in 15.2% (n=5) of the samples. Toxocara cati was present in 3.0% (n=1) of the poultry chickens. The PCR assay in this study is a specific and rapid alternative method for identification of Toxocara larvae and allowes the observation of specific species in the field conditions within the locations where these chickens are typically raised.

Keywords: Toxocara, broiler chickens, PCR,

# THE TIGER TOOTH CROAKER (OTOLITHES RUBER), A PARATENIC HOST OF THE ZOONO-TIC ANISAKID NEMATODES FROM PERSIAN GULF

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Persian Gulf located in the south and south east of Iran, has a vast potential of marine fish commercial production. The tiger tooth croaker, Otolithes ruber, is one of the most important commercial fish species in Persian Gulf which can act as intermediate, paratenic or definitive host of the anisakid nematodes. In humans, the ingestion of their third-stage larvae through consumption of lightly cooked or raw infested fish can causea zoonotic disease known anisakiasis and these larvae invade the gastrointestinal mucosa and lead to abdominal pain, fever, vomiting, nausea, diarrhea, eosinophilia and different gastrointestinal lesions. Those that cause anisakiasis are larvae of Anisakis sp. in most cases followed by larvae of Pseudoterranova sp. Other anisakid larvae, such as Contracaecum sp. and Hysterothylacium sp. are hardly ever found in humans. The present study was performed to evaluate the nematode infection rate with emphasis on the zoonotic interest in O. ruber from the Persian Gulf. A total of 120 specimens of O. ruber with the total length of 25-57 cm and weight of 295-565 g from three sampling sites (Khuzestan, Bushehr and Hormozgan) of the Persian Gulf were studied from August 2012 to December 2013. Their abdominal cavity was washed under running water into a 50 mesh sieve and examined microscopically. The nematode larvae were fixed in 70% ethanol, cleraed in lactophenol for 48h, their schematic drawing were prepared. Identification was carried out using available keys of Yamaguti and Gibbons, prevalence and intensity were calculated according to Bush et al. and data analysis were carried out using SPSS, version 16. T-test was used to determine the relation between size of fish and parasitic intensity, at significance level of 0.05. Out of the 120 examined specimens, 25 (20.83%) were parasitized with anisakid larvae. Identified genera, Anisakis sp., Raphid Ascaris sp. and Contracaecum sp. had the prevalence of 5.2 %, 3.8 % and 2.5%, respectively, being isolated from liver and abdominal cavity. There were significant differences between intensity of infestation and size of infected fish and intensity of infestation was increased with the size of the host (P <0.05).No human anisakiasis is yet recorded from Iran, this issue could be attributed to cuisine habit of fish in studied areas as well as other parts of the country, but with an increasing trend to use undercooked or raw fish among Iranians, the prevalence of anisakiasis cases is expected to merge. Since anisakiasis is considered as an emerging zoonosis, anisakid larvae require particular attention of investigation of their morphology, biology and life cycle.

Keywords: Otolithes ruber, Anisakid larvae, zoonotic nematodes, anisakiasis





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# INVESTIGATION OF PARASITIC CONTAMINA-TION IN ORNAMENTAL FISH, BLUNTHEAD CICHLID (TROPHEUS MOORII)

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Blunthead cichlid (Tropheus moorii) is one of the most popular freshwater ornamental fish from Cichlidae family that its breeding has recently become popular among breeders in our country. This fish is an imported aquarium fish, which often imported as fry that reared and supplied by breeders in Iran. The purpose of this study was to investigate the causes of mortality of blunthead cichlid in an ornamental fish farming center in Karaj. In November of 1392 in aquarium fish farming in Karaj city, symptoms like lethargy, anorexia and seclusion were observed in imported blunthead cichlid fish. Fish had entered to Iran, 20 days before the clinical signs appeared. To determinate the agents of this event, 3 live fry were referred to ornamental fish clinic in Faculty of Veterinary Medicine of University of Tehran. Then wet smear were prepared from skin, fins and gills of the fish were examined under a light microscope. Internal organs such as intestine were examined for parasitic infections. No parasites were seen in microscopic examination of the skin and gills of fish. In examining the internal organ, *Camalanus* sp. (Nematoda) was observed. Infection intensity with Camalanus sp. was a relatively high and the main factor of the fish problem was involvement with this parasite. The rest of the sick fish, treated twice with antiparasitic drug. Then 1 and 2 weeks after second treatment, the number of fish were randomly necropsied and investigation of parasites was done. After treatment, microscopic studies showed that parasitic infections were completely resolved and no parasitic infection in the intestine were observed. After a week of starting treatment, mortality was completely cut off.

Keywords: parasitic contamination, *Camalanus* sp., blunthead cichlid

# PREVALENCE OF DOGS' INFECTION WITH DIROFILARIA IMMITIS USING SOME LABORA-TORY DIAGNOSTIC METHODS, IN URMIA, NORTHWEST IRAN

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Dirofilariasis also called canine heartworm disease (HWD) is a prevalent disease in Iran, particularly in northwest temperate zones. Because of some limitations due to religious believes there are no enough cares about the dogs' health. This study aimed to evaluate the prevalence rate of the disease and compare the potency of some available laboratory diagnostic tests in dogs referred to small animal veterinary clinics in Urmia, northwest Iran. Eighty blood samples were collected from clinics, which had been obtained from dogs with no apparent signs of HWD for different hematology/ biochemistry tests from June to November 2011. Consequently, the samples were examined using a sandwich immunochromatographic Kit as the gold standard and Modified knott test and direct smear test as the frequent diagnostic tests in veterinary clinics. The results showed 12.5% infection in the studied samples. Also, despite the fact that, HWD was more frequent in 4-7 year age group (20%), male dogs (14.3%), outdoor kept (15.2%) and in bad sanitary condition dogs (16.1%), comparing to 1-4 year and more than 7 years age groups (5.9%, 12.5%), female ones (8.3%), indoor (0%) and appropriate sanitary condition (0%), respectively. Statistical analysis showed no significant relationship between the disease and the studied parameters (Chi square test- P> 0.05). Comparison of the other tests' results with the above mentioned kit results revealed the substantial agreement between kit and modified knott test (Kappa value= 0.787). It can be concluded that HWD is prevalent in Urmia and both kit and modified knott tests can be used as appropriate methods according to available facilities, but it should be considered that about 40% of the infected animals have the occult infection which can complicate the clinical outcomes of the modified knott test diagnostic results.

**Keywords:** dirofilariasis, laboratory diagnosis, Dog, Urmia, Iran





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# ZOONOTIC ANISAKID NEMATODES OF HY-GIENIC IMPORTANCE INFECTING THE NAR-ROW-BARRED SPANISH MACKEREL (SCOMBEROMORUS COMMERSON) IN THE PERSIAN GULF

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Scomberomorus commerson is one of the most important commercial species in Persian Gulf. The anisakid nematodes were reported from several marine fish. Humans can accidentally be infected by the ingestion of their 3 larvae belonging to the genera Anisakis, Pseudoterranova, Contracaecum and Hysterothylacium in raw or undercooked seafood. These larvae cause a zoonotic disease known as anisakiasis. The present study was undertaken to investigate the possibility of S. commerson acting as a paratenic host for these zoonotic larvae from Persian Gulf. A total of 120 specimens of S. commerson with the total length of 30-195 cm and weight of 350-7000 g were studied from August 2012 to December 2013. Obtained larvae were fixed in 70% ethanol, cleraed in lactophenol for 48h and identified using available keys of Yamaguti and Gibbons. Prevalence and intensity were calculated according to Bush et al., data analysis was carried out using SPSS 16 and t-test was used to determine the relation between size of fish and parasitic intensity. The overall prevalence was 8.33 %. Identified genera, Hysterothylacium sp. and Anisakis sp. had the prevalence of 5.8% and 4%, respectively, being removed from intestine, stomach and abdominal cavity. There were significant differences between intensity of infestation and size of host and intensity was increased with their size (P <0.05). Anisakiasis is considered as an emerging zoonotic disease. These worms in the final hosts and also in humans cause serious gastrointestinal illness and even death in heavy infections so their presence is a great concern from a public health point of view and increases the necessity to provide more information about ichthyo parasitoses and prophylactic approaches at various levels.

Keywords: Scomberomorus commerson, anisakid larvae, zoonotic nematodes, anisakiasis

# A COMPARISON SEROPREVALENCE STUDY OF TOXOCARIASIS IN DOG AND CAT OWNERS AND CONTROL GROUP IN MASHHAD BY ELISA METHOD

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Toxocariasis is the clinical terms applied to infection of human with animal ascarid nematodes, Toxocara canis and T. cati. Because of the increasing interests of people for keeping pets (cats and dogs), and due to paucity of seroepidemiological study of toxocariasis in Mashhad, we decided to determine the seroprevalence of toxocariasis among people who keep cats and dogs. A serological study for detection of antibodies to Toxocara in two groups (93 cat and dog owners referred to veterinary clinic of Ferdowsi University of Mashhad as case group and 93 healthy people as control group) was performed from Feb 2013 to Dec 2013. An ELISA method was employed using determination of IgG antibodies against Taxocara obtained in parasitology and immunology lab of Imam Reza hospital of Mashhad. Using a questionnaire, epidemiological factors associated with infection were determined. The seroprevalences of Toxocara antibodies in the pet owners and control group were respectively 20.43% (19/93) and 1.07% (1/93) respectively. Mean age of pet owners was 32.23±10.25 and of control group 37.59 11.62±. Concerning the gender of studied population, 41.9% of pet owners were female and 58.1% male.Seroepidemiological studies carried out in Iran revealed seroprevalence rates of 2% to 25.6% among the normal human population. Unfortunately, there is not any information among persons exposed to those parasites (i.e. pet owner, veterinarians, and farmers). In this study for the first time, we found the seroprevalence of toxocariasis in pet owners in Mashhad, Iran. The data show the significant difference between seroprevalence of Toxocara among pet owners and control group. General practitioners, veterinarians, and public health organizations should provide sufficient information for preventing contamination of people and environment as well.

Keywords: visceral larva migrans, dog, cat, ELISA method





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ENTEROBIUS VERMICULARIS INFECTION AMONG KINDERGARTENS AND PRESCHOOL CHILDREN IN MAZANDARAN PROVINCE, NORTH OF IRAN

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Enterobiasis (Oxyuriasis) is probably the most common intestinal helminthic disease of humans. Prevalence of Enterobius vermicularis, the causal agent of enterobiasis, is higher in children than the adults. This nematode can result in loss of appetite, insomnia, pruritus ani, grinding of the teeth, restlessness, endometritis, abdominal cramps, diarrhea in children.Due to important complications of this parasite, the objective of the current study was to determine the prevalence of enterobiasis in kindergarten and preschool children of Amol and Amir Kola of Babol, Mazandaran province, north of Iran in 2013. A total of 588 children from kindergartens and preschool of Amol and Amir Kola were examined for the prevalence of E. vermicularis infection. Adhesive cellotape anal swab method was trained to parents for sampling. In addition, a questionnaire was designed and filled out to collect demographic information for each individual. Data were analyzed using SPSS software. The overall prevalence of E. vermicularis infection was 10.3% (61). Although infection with E. vermicularis in girls 5.4% (32) was higher than boys 4.9% (29), no statistically significant difference was observed between two genders and age groups (p > 0.05). The findings indicated relatively high infection rate of E. vermicularis in kindergarten and preschool children in study areas. Due to its contagious nature enterobiasis should be considered as an important health problem in children and implementation of educational programs will play a crucial role in decreasing the infection rates in local children.

Keywords: prevalence, *Enterobius vermicularis*, children, school, intestinal infection

# AN INVESTIGATION OF HORMONAL CHANGES IN WOMEN WITH BREAST CANCER INFECTED WITH TOXOPLASMA GONDII

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Toxoplasmosis is a parasitic infection seen in many species of mammals and humans that occurs congenitally and is acquired in chronic and acute forms in humans. Acute Toxoplasmosis is more critical in immunocompromised patients with AIDS or cancer and also people consuming immunosuppressive drugs. The present study was conducted to investigate the anti-Toxoplasma gondii antibodies and assess the toxoplasmosis risk in women with breast masses in cities of Kermanshah and Ilam in 2011. This research was done as a case-control study on women referred to the treatment centers in cities of Kermanshah and Ilam in 2011. Sixty women with breast cancer were selected as cases, and 60 healthy ones as the control group. They were evaluated in terms of level of IgG, IgM, estrogen and progesterone using ELISA method. Women in this study were in the secretory phase and were under the age of menopause. Data were analyzed using SPSS software 16 with independent T test. Results of this study showed a significant relationship between Toxoplasma gondii infection and breast cancer (P<0.01). There was a significant relationship between anti-Toxoplasma IgG antibody and breast cancer (P=0.01). Women with breast cancer and healthy women were not reported positive in terms of IgM anti-Toxoplasma. Significant relationship was detected between estrogen and Toxoplasma in women with breast cancer (P < 0.01). There was no significant relationship between the level of progesterone and anti-Toxoplasma antibodies. A total of 30 patients (50%) from studied women had tumor marker CEA 19-9 (Carcinoembryonic antigen).Patients with breast tumors may be at risk of opportunistic infections such as toxoplasmosis. Therefore, periodic review of breast cancer patients should be conducted by clinicians to prevent the potential occurrence of toxoplasmosis.

**Keywords:** : *Toxoplasma gondii*, breast cancer, toxoplasmosis, Iran





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# SEROPREVALENCE OF TOXOPLASMA GONDII INFECTION IN 12 TO 19 YEARS OLD GIRLS IN AJABSHIR, IRAN

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Toxoplasmosis is a parasitic disease with various prevalences in different countries. Some experts consider this parasitic disease as a cause of abortion. The aim of this study was to determine the prevalence of anti-Toxoplasma antibodies among high school girls in the villages and the city of Ajabshir. A descriptive study was conducted on 549 female students who were divided into two groups. (147 from urban and 402 cases from rural areas). Blood samples were taken from all subjects. IgG and IgM antibodies were analyzed by ELISA. This study showed that 12.4% of the urban population and 25.9% of the rural population are positive for IgG. Also 8.5% of the urban population and 21.8% of the rural population were positive for IgM. This difference was statistically significant (p<0/001). The study found that 87.6% of women surveyed did not have antibodies to Toxoplasma. So in order to prevent the risk of infection during pregnancy, especial education and health measures should be implemented.

Keywords: toxoplasmosis, ELISA, seroprevalenc, Iran

IN VIVO EFFICACY OF BUNIUM PERSICUM SEEDS TO TREAT ACUTE TOXOPLASMOSIS IN MICE

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Toxoplasmosis, caused by the protozoan parasite Toxoplasma gondii, is one of the most common parasitic infections of man and other warm-blooded animals. At present, the first choice drugs to treat toxoplasmisis have some limitations in use. The present study aimed to evaluate the in vivo activity of Bunium persicum Boiss essential oil on infected mice with acute toxoplasmosis. In order to investigate treatment effects of B. persicum oil against acute toxoplasmosis in mice, at first, 2 groups of mice were infected intraperitonealy with 104 tachyzoite of T. gondii, RH strain. After 24 h. a group of mice received B. persicum oil at concentrations of 0.05 ml/kg twice a day for 5 days. Another group of mice received B. persicum oil at concentrations of 0.1 ml/kg twice a day for 5 days. The control received olive oil for 5 days. The mortality rate (time of death) in all infected mice were recorded. Furthermore, the number of parasites (tachyzoites) isolated from infected mice were counted under light microscope. Results demonstrated that mortality rate of infected mice was 6 and 7 days after oral administration of B. persicum oil at concentration of 0.05 and 0.1 ml/kg, respectively (P<0.05). In contrast, the mortality rate in control group was 5 days. In addition, mean number of tachyzoites was  $189 \times 104$  and  $133 \times 104$  for infected mice treated with 0.05 and 0.1 ml/kg, respectively (P<0.05); while, in control group, the mean number of tachyzoites was 412×104 parasite. The obtained finding revealed the potential of B. persicum oil as a natural source for the production of new agent to treat acute toxoplasmosis. However, further studies will be needed to confirm these results by checking the essential oil and its active component in a clinical setting as a new agent.

Keywords: Toxoplasma gondii, toxoplasmosis, Bunicum persicum, Iran





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# PROPHYLACTIC EFFECTS OF THE BUNIUM PERSICUM (BOISS) ESSENTIAL OIL AGAINST ACUTE TOXOPLASMOSIS IN MICE

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Toxoplasmosis, caused by the protozoan parasite Toxoplasma gondii, is one of the most common parasitic infections of man and other warm-blooded animals. At present, the first choice drugs to treat toxoplasmisis have some limitations in use. The present study was aimed to evaluate the in vivo activity of Bunium persicum Boiss. essential oil on infected mice with acute toxoplasmosis. To evaluate prophylactic effects of B. persicum oil on acute toxoplasmosis, male mice received B. persicum oil at concentrations of 0.05 and 0.1 ml/kg for 14 days. The control group of mice received olive oil for the same period. After 24 h mice were infected intraperitonealy with 104 tachyzoites of T. gondii, RH strain. The mortality rate in all infected mice was recorded. In addition, the number of parasites (tachyzoites) isolated from infected mice were counted under light microscope. Findings revealed that mortality rate of infected mice was 8 and 9 days after oral administration of B. persicum oil at concentration of 0.05 and 0.1 ml/kg, respectively (P<0.05). In contrast, the mortality rate of control group was 5 days. In addition, mean number of tachyzoites was  $192 \times 104$  and  $64 \times 104$  for infected mice treated with 0.05 and 0.1 ml/kg, respectively (P<0.05); while, in control group, the mean number of tachyzoites was 288×104 parasite. The results showed the potential of B. persicum oil as a natural source for the production of new prophylactic agent for use in toxoplasmosis. However, further studies will be needed to confirm these results by checking the essential oil and its active component in a clinical setting as a new prophylactic agent.

Keywords: Toxoplasma gondii, mice, in vivo, RH strain, Bunium persicum

# EVALUATION OF CELL IMMUNITY RESPONSES AGAINST LYSATE AND EXCRETORY-SECRETARY ANTIGENS FROM TOXOPLASMA GONDII: AN IN VITRO STUDY

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Obligate intracellular protozoan, Toxoplasma gondii, that causes toxoplasmosis, has a global emission in the warm-blooded vertebrates including humans. It occurs in two clinical forms, acquired and congenital. The frequency of toxoplasmosis seropositive individuals is not less than 30 present in any place on the Earth. Furthermore, more than 25 percent of deaths in HIV infection are due to cerebral toxoplasmosis. If the parasitemia infection is acquired in pregnancy, abortion, premature birth, or severe neonatal eye and brain lesions may occur. In this study, we aimed to evaluate cell immunity responses against Total and Excretory-Secretary Antigens from Toxoplasma gondii in vitro. Approximately 45 × 106 live tachyzoites of the RH strain of T. gondii were used for preparation of Toxoplasma lysate and excretory-secretary antigens, then isolation of Peripheral Blood Mononuclear Cells (PBMC) was performed. These cells were isolated by blood centrifugation on Ficoll-Paque. We cultured 2 ×106 cells per well per ml in a 24-well microplate in the presence of 5  $\mu$ g/ml PHA, 5  $\mu$ g/ml lysate and 5  $\mu$ g/ml excretory-secretary Antigens. Plates were incubated at 37°C in a 5% CO2 atmosphere. Supernatants were collected at 24 and 96 h. The supernatants were then centrifuged, and stored at -70°C for analysis of cytokine production. Data are expressed as mean  $\pm$  SD of IFNy and IL4 (pg/ml) Secretion in the supernatant of PBMC stimulated Toxoplasma lysate and excretorysecretary, and were statistically analyzed by ANOVA test for comparing the variables among groups. Concentration mean of IL4 (pg/ml) Secretion in the supernatant of PBMC stimulated Toxoplasma lysate was 42.12±2.27, excretory-secretary was 32.20±0.35, both Toxoplasma lysate and excretorysecretary was 38.00±0.23 and PHA was 98.00±1.04.The mentioned difference was statistically significant (P<0.001). Also concentration mean of INF  $-\gamma$  (pg/ml) secretion in the supernatant of PBMC stimulated Toxoplasma lysate: 132.3±1.11, excretory-secretary: 185.0±2.16, both Toxoplasma lysate and excretory-secretary: 208.6±1.42 and PHA: 883.9±14.21. The mentioned difference was statistically significant (P<0.001). Toxoplasma lysate and excretory-secretary play an important role in control of the infection as the in vitro experiments demonstrated that they participate in the stimulation of Peripheral Blood Mononuclear Cells. Such antigens might be included in a future vaccine against toxoplasmosis.

Keywords: *Toxoplasma gondii*, cell immunity, excretory-secretary antigens





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APPLICATION OF LOOP-MEDIATED ISOTHER-MAL AMPLIFICATION (LAMP) TECHNIQUE AND COMPARISON WITH NESTED PCR ASSAY FOR DETECTION OF TOXOPLASMA GONDII

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Toxoplasmosis diagnosis constitutes an important measure for disease prevention and control. In this paper, a newly described DNA amplification technique, loop-mediated isothermal amplification (LAMP), and nested PCR targeting, the two commonly used genomic repeats of Toxoplasma gondii, RE and B1, were compared to each other for the diagnosis of toxoplasmosis in children with blood cancer. One hundred and ten DNA samples of these patients were analyzed by LAMP and nested-PCR. The analytical sensitivity of assays was evaluated by 10-fold serial dilutions of T. gondii DNA ranging from 1 ng to 0.01 fg. DNA samples of other parasites and human chromosomal DNA were used to determine the specificity of molecular assays. In order to determine the early diagnosis potency of LAMP and nested-PCR assays for T. gondii infection in mice, blood samples taken on different days from infected mice were analyzed with RE, B1- LAMP and RE, B1-nested PCR assays. Out of 50 seropositive (IgM+, IgG+) samples, positive results were obtained with 92% (46/50) and 86% (43/50) on RE, B1-LAMP assays and 82% (41/50) and 68% (34/50) on RE, B1-nested PCR analyses, respectively. Of the 50 seronegative samples, three (6%) samples by RE-LAMP, two (4%) samples by B1-LAMP and one (2%) sample by RE-nested PCR assay were detected positive while none were detected positive by B1-nested PCR assay. The detection limits of the RE, B1-LAMP and RE, B1nested PCR assays were one fg,100fg,one pg and 10 pg of T. gondii DNA respectively. No cross-reactivity with the DNA of other parasites and human chromosomal DNA was found. This is the first report of a study in which the LAMP method was applied to diagnose toxoplasmosis in blood samples of children with leukemia. The RE-LAMP assay provided the most sensitive diagnosis of toxoplasmosis in children with leukemia. Nevertheless, the B1-LAMP assay also has potential as a diagnostic tool for detection of T. gondii in cancer patients.

Keywords: LAMP, Nested-PCR, *Toxoplasma gondii*, children, cancer

CASE-CONTROL STUDY OF THE ASSOCIATION BETWEEN TESTOSTERONE LEVELS AND SE-RUM LIPIDS IN HEALTHY MEN AND MEN WITH CHRONIC TOXOPLASMOSIS

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Toxoplasma gondii is one of the most infective parasites in the world. It is capable to infect all warm-blooded animals. It has been estimated that about one third of the world population are infected with this parasite. This parasite can cause severe infection and abortion. There is evidence that host lipids are involved in the pathogenesis of Toxoplasma. Therefore, this study measured and compared lipid and testosterone levels of serum in men infected with toxoplasmosis and non- infected men. This case - control study was conducted on 200 men, 20 to 29 years old, admitted to the post marriage center. After completing a questionnaire, a blood sample (5 ml) was taken from each subject. Then, required diagnostic tests for toxoplasmosis was conducted. According to diagnostic results, 100 men with Toxoplasma antibodies were defined as cases and 100 others without antibodies as controls. Then, levels of testosterone and lipids in serum of these two groups were measured and compared. Results showed that testosterone levels were normal in case and control groups but the hormone level in the case group was significantly lower than the control group. The study of relationship between testosterone level and serum lipids in case and control groups showed a significant negative correlation between testosterone level and triglyceride level. Also there is a significant positive correlation between testosterone level and HDL levels.

Keywords: testosterone, toxoplasmosis, serum lipids, men





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SEROPREVALENCE OF IGG AND IGM IMMUNO-GLOBULINS AGAINST TOXOPLASMA GONDII IN PREGNANT WOMEN AND UMBLICAL CORD OF THEIR INFANTS BORN IN RAFSANJAN IN 2013

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Toxoplasma gondii infects a wide variety of vertebrates, as well as 30-60 percent of humans in many countries including Iran. The present study aimed to estimate the infection prevalence in pregnant women and incidence of congenital toxoplasmosis in newborns and to determine its association with some potential epidemiological factors. Serums of 333 blood samples of pregnant mothers and their infants' umbilical cords were isolated. Serum samples were examined for IgG and IgM Immunoglobulins against Toxoplasma gondii using commercial ELISA kits. Data on some potential epidemiological parameters of infection were recorded. 73 out of 240 (30.4%) mother blood samples and 83 out of 254 (32.7%) umbilical cord samples had detectable IgG antibodies against Toxoplasma gondii. On the other hand, out of 266 pregnant mother and umbilical cord blood samples, only one (0.3%) umbilical cord sample was positive for IgM immunoglobulins. Positive IgG results had statistically significant associations with job, educational levels, residence in rural or urban area and close contact with cats. Odds ratio of infection in housewives was 3.8 times of non-housewives, in rural women was 2.24 times of urban women, and in those with close cat contacts was 1.84 times of those without contact.In spite of being situated near Lut desert and having a warm and arid climate, Kerman province has suitable conditions for Toxoplasma infection transmission through its three main routes of cats, meat and placenta. Living in rural area, close contact with cats, being housewife and lower literacy are the main risk factors for infection acquisition.

Keywords: *Toxoplasma*, congenital toxoplasmosis, umbilical cord, Rafsanjan, Iran

# MOLECULAR DETECTION OF TOXOPLASMA GONDII INFECTION IN ABORTED FETUSES IN SHEEP, IN KHORASAN RAZAVI PROVINCE, IRAN

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Toxoplasmosis caused by the protozoan parasite namely Taxoplasma gondii that infects all of warm- blooded animals and humans. This parasite is transmitted to sheep via ingesting food and water contaminated with oocysts which are excreted by cats or by transmission from mother to fetus (congenital toxoplasmosis). Toxoplasmosis causes reproduction disorders such as fetal resorption, early embryonic death, mummification, abortion, still birth, neonatal and fetal death in sheep. Toxoplasmosis makes significant economic loss due to induction of abortion in sheep and goat worldwide. Various techniques have been applied for diagnosis of abortion caused by toxoplasmosis in infected sheep. In the previous studies, the diagnosis of T. gondii infection was usually based on serological assay, histopathological examination, and isolation of Toxoplasma by mouse inoculation while in the recent studies molecular methods are used. The aim of this study was to determine the frequency of Toxoplasma infection in aborted ovine fetuses in the province using molecular methods. 112 brain samples of aborted fetuses of sheep were collected from 2009 to 2014 and were examined to detect Toxoplasma gondii infection by the Nested-PCR technique. Result: The result showed that 18 out of 112 (16.07%) ovine fetuses' brain samples were PCR-positive based on the Nested PCR. In this study, a significant difference was found between Toxoplasma infections in two different area of Khorasan province (≤P0.05).DNA detection of Toxoplasma gondii by molecular methods is more sensitive than other methods. Isolation and detection DNA of Toxoplasma gondii in aborted fetuses of sheep shows that this parasite is important in abortion. The most positive samples were detected in aborted fetuses in the north areas of Khorasan Razavi province which have more humidity than the south areas with dry climate. Higher prevalence rate of toxoplasmosis in moist areas compared arid areas is related to the longer viability of T. gondii oocysts in moist. The results of our study show that congenital toxoplasmosis in sheep is extremely important because infected sheep play an important role as source of human infection.

Keywords: *Toxoplasma gondii*, abortion, sheep, Nested-PCR, Iran





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# SEROPREVALENCE OF TOXOPLASMA-SPECIFIC ANTIBODIES IN PATIENTS SUSPECTED TO HAVE ACTIVE TOXOPLASMOSIS: A CROSS-SECTIONAL SURVEY

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The aim of this study was to investigate the presence and distribution of anti-Toxoplasma specific IgM and IgG antibodies in patients suspected to have toxoplasmosis and investigate for any association between IgM and IgG antibodies and some toxoplasmosis risk factors as well. In a comparative cross-sectional study, 70 patients suspected to have active toxoplasmosis and 30 control volunteers entered the study. In each group, patient age, sex, signs on the appearance, education level, residency status (urban / rural), occupation, frequency of Toxoplasma -specific IgG and IgM antibodies, abortion history, and some risk factors (direct cat exposure, occupational exposure to raw meat, and raw vegetable consumption) were recorded. The enzyme-linked immunosorbent assay (ELISA) kits (EUROIMMUN®, United Kingdom) were used for the evaluation of anti Toxoplasma IgG and IgM antibodies according to the manufacturer instructions. All analyses were done using SPSS-20. The frequency of Toxoplasma -specific IgG and IgM antibodies and the mentioned risk factors were not statistically significant between the two groups (P > 0.05). The history of previous abortions in women in the toxoplasmosis-suspected group was significantly higher than that in the controls (31.4% versus 6.7%; P = 0.009). The frequency of specific IgM and IgG antibodies in suspected toxoplasmosis and control groups was not statistically significant.

Keywords: IgG, IgM, antibody, Isfahan, Toxoplasma gondii

# EFFECT OF WATERY EXTRACT AND ALCOHOL-IC NIGELLA SATIVA ON TOXOPLASMA GONDII TACHYZOITE IN-VITRO

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Blood and tissue protozoans are able to cause serious dieases such as toxoplasmosis in humans. The aime of this study was to investigate the use of herbal drugs as substitute for chemical drugs, considering the drug resistance and side effects of the later. Toxoplasma gondii tachyzoite isolalated from infective small white mice preitoneum were used. Alcoholic extracts of Nigella sativa were added to them in concentration of to 10 ml, 20 ml, 50 ml, 100 ml, 200 ml, in 24 hours, 48 hours, 72 hours and then results investigated using Neobar slide and light microscope. The results showed that Nigella sativa alcoholic extract with concentration of 200 ml in 48 hours and Nigella sativa watery extract with proportion 200 ml in 72 hours caused remarkable reduction effect in the number of Toxoplasma gondii tachyzoite. Nigella sativa alcoholic and watery extracts have anti protozoan effect. More extensive studies are recommended to prove this issue.

Keywords: Toxoplasma gondii, alcoholic extract, Nigella sativa





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# DIAGNOSIS AND COMPARISON OF ACUTE AND CHRONIC TOXOPLASMOSIS IN YOUNG COU-PLES ATTENDING PREMARITAL LABORATORY IN ARAK CITY

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Toxoplasmosis is an important zoonotic parasitic disease worldwide. In immune competent individuals, Toxoplasma gondii preferentially infects tissues of central nervous system. Congenital transmission of T. gondii during pregnancy has been regarded as a risk factor for the health of newborn infants. The aim of this study was to diagnose and compare acute and chronic toxoplasmosis in young couples attending premarital laboratory in Arak. In this experimental study, 100 young couples attending premarital laboratory were included. Serum specimens were prepared and tested for Anti-T. gondii IgM and IgG. Statistical analysis were carried out by using the SPSS software version 16. Out of a total of 100 women tested, 5 cases were positive for IgM antibody and 36 cases were positive for IgG antibody. All of the men's samples were negative for IgM antibody, but 21 samples of men were positive for IgG antibody. The comparison between positive and negative cases among men and women showed significant differences between them in anti-T. gondii IgM positive cases (p=0.024). The comparison between positive and negative cases among men and women showed significant differences in anti-T. gondii IgM positive cases (p= 0.017). It was observed that about 61 percent of women and 79 percent of men had no antibodies against T. gondii, so these cases have no immune protection against this parasite. This is especially important for women of childbearing age and should avoid infection sources in pregnancy period.

Keywords: antibody, premarital, laboratory, Toxoplasma gondii

# IDENTIFICATION OF TOXOPLASMA GONDII IN SHEEP TISSUE SAMPLES USING NESTED-PCR

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Toxoplasma gondii is an intracellular parasite, which is widely prevalent in humans and warm-blooded animals throughout the world. Infection can occur pre- or post-natally. Humans are usually infected with T. gondii by ingestion of oocysts in soil or water contaminated with cat feces, or by ingestion of tissue cysts in undercooked meat. Furthermore, T. gondii is an important cause of abortion and stillbirth in sheep and is a significant cause of economic loss to sheep industry. The current study was conducted to identify the presence of T. gondii DNA in tissue samples from slaughtered sheep in Khorramabad, southwest of Iran. Brain samples and meat samples (containing Diaphragm, tongue and masseter muscles) were collected from each examined sheep. Totally 60 tissue samples were collected from 30 slaughtered ewes. DNA extraction was performed using a DNA isolation kit (MBST, Iran). A nested-PCR which targets the B1 gene has been used for tissue samples. Tissue samples were considered T. gondii-positive if the expected band size for the second round (94bp) appeared. Results of B1-PCR revealed the presence of T. gondii in 76.6% of the examined animals. The parasite was detected in 21 (70%) brain samples and 8 (26.6%) meat samples. Dual contamination was detected in 6 (20%) ewes. Due to variations in weather, cat populations and diagnostic methods, there is a wide variation in T. gondii prevalence within different regions of Iran. These results indicate that a considerable proportion of sheep meats and brains are contaminated with T. gondii, which has important implications in public health. Furthermore, the high prevalence of infection among ewes indicate that T. gondii might be considered as one of the major causes of ovine abortion in this region.

Keywords: Toxoplasma gondii, Nested-PCR, tissue samples, sheep





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MOLECULAR DIAGNOSIS OF RE AND B1 GENE FOR DETECTION OF TOXOPLASMA GONDII IN-FECTION IN DIABETIC PATIENTS BY NESTED-PCR

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Toxoplasma gondii is an obligate intracellular protozoan parasite with a worldwide distribution. Several techniques have been developed for T. gondii detection. In this study RE and B1genes of Toxoplasma gondii were used and compared to each other in nested-PCR assay. 945 blood samples from people referred to the Diabetic center of Ali Asghar hospital in Zahedan were collected. Two hundred and five DNA samples of selected diabetic subject confirmed by the physician were tested for the presence of T. gondii antibodies using enzyme immunoassays using nested-PCR. The data was analyzed by SPSS.ver 18. Chi-square test was used for comparison. Out of 205 diabetes patients, 53 cases were (IgG+, IgM+), 20 (IgG-, IgM+), 72 (IgG+, IgM-) and 60 cases were (IgG-, IgM-). The Nested-PCR detected that acute group 21/53 (39.63%), 30/53 (56.60%) (IgM+, IgG+), chronic group 40/72 (55.56%), 51/72 (70.83%) (IgG+, IgM-), false positive 18/20 (90%), 17/20 (85%) (IgM+, IgG-), and seronegative samples 38/60 (63.33%), 60/ 41(77.35%) for RE and B1 gene respectively. The prevalence of toxoplasmosis in patients with diabetes to B1 gene 139 (67.8%) and RE 117 (57.1%) were positive. Our study demonstrated that B1 more than RE gene showed positive samples and can be used to detect, although B1 in comparison with RE gene didn't have any superior molecular diagnosis.

Keywords: RE, B1, Nested-PCR, Toxoplasma gondii, diabetic patient

ASSOCIATION BETWEEN TOXOPLASMOSIS AND SPONTANEOUS ABORTION AND STILLBIRTH

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Congenital toxoplasmosis is one of the most important infectious diseases that may causes abortion. There is limited information about the role of congenital toxoplasmosis in the etiology of abortion and stillbirth in pregnant women. The aim of this study was to evaluate the role of toxoplasmosis in the etiology of spontaneous abortion and stillbirth based on molecular and serological techniques. In this case control study 110 pregnant women with spontaneous abortion and stillbirth enrolled as case group, and 110 pregnant women with normal delivery in maternity hospitals of Tehran enrolled as control group. Serum samples of both groups were tested for anti- Toxoplasma IgG and IgM antibodies by ELISA method. Placenta samples of both groups were taken for detection of T. gondii DNA by Polymerase Chain Reaction. A questionnaire including demographic data, risk factors, and clinical symptoms were recorded from case and control groups. The seroprevalence of IgG was 25.5% in case group and 26.4% in control group (p=0.8). IgM seropositivity was detected in 2.7% of case group and 0.9% of control group (p=0.37). T. gondii DNA was detected in 6.4% of case and 1.8 % of control groups by PCR (p=0.17). The major risk factor of congenital toxoplasmosis was the history of eating undercooked meat (p=0.06). The results of this study showed that the rate of PCR positive in women with abortion and stillbirth was 3.7 times more than controls, but the difference was not statistically significant. These findings suggest that toxoplasmosis can be involved in the etiology of abortion and stillbirth, however, more research with larger samples is recommended in this area.

Keywords: Toxoplasma gondii, PCR, ELISA, abortion





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A STUDY ON TOXOPLASMA GONDII AND NE-OSPORA CANINUM INFECTION IN SHEEP IN MASHHAD AREA, KHORASAN RAZAVI PROV-INCE

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Toxoplasma gondii and Neospora caninum belong to Sarcocystidae family. Life cycles of them are very similar and they are capable to infect a wide range of animals in the world. T. gondii is a main causative of abortion in sheep, while the role of N. caninum infection in causing abortion in sheep is unknown and need more investigation. The aim of study was to compare the rate of T. gondii and N. caninum infection in sheep in Mashhad area. From February to December 2014, 30 head of sheep were monthly purchased from Torghaba abattoir. The sheep skull was opened and brain tissue removed for microscopic and molecular examination. First, Impression smears were prepared from different parts of brain areas and stained with Giemsa staining and brain tissue samples were collected for DNA extraction and bioassay examination. Specific PCR assay was performed to detect T. gondii and N. caninum DNA in brain samples. The brain tissues of PCR-positive samples were bioassayed in the Outbred Swiss Webster and BALB/C mice. The mice were bled and tested for T. gondii or N. caninum antibodies 5 weeks p.i by IFAT. The seropositive mice were also euthanized and then stained impression smears of brain were prepared and microscopically examined for tissue cysts observation. In the present study, the cyst and zoites of T. gondii were not microscopically observed in any stained smears of brain samples of sheep. T. gondii DNA was detected in 13 (43%) brain tissues samples of sheep by nested-PCR. Two groups of inoculated mice developed antibodies against T. gondii and tissue cysts were found in the brain smears of some mice of infected group. N. caninum infection was not detected in any brain samples of sheep by PCR.Our results show that T. gondii infection is common in the sheep of Mashhad area and it seems the N. caninum infection rarely occurs in sheep.

Keywords: Toxoplasma gondii, Neospora caninum, sheep, Mashhad

# SEROPREVALENCE OF TOXOPLASMA GONDII IN IRANIAN GENERAL POPULATION: A SYS-TEMATIC REVIEW AND META-ANALYSIS

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Toxoplasma gondii is one of the most common protozoan parasites with widespread distribution globally. It is the causative agent of Toxoplasma infection prevalent in human and other warm-blooded vertebrates. While T. gondii infection in healthy people is usually asymptomatic, it can lead to serious pathological effects in congenital cases and immunodeficient patients. We sought to identify the seroprevalence rate of Toxoplasma infection in the Iranian general population to develop a comprehensive description of the disease condition in Iran for future use. Electronic databases (PubMed, Google Scholar, Science Direct, and Scopus) and Persian language databases (Magiran, Scientific Information Database [SID], Iran Medex, and Iran Doc) were searched. Furthermore, graduate student dissertations and proceedings of national parasitology congresses were searched manually. Our search resulted in a total of 35 reports published from 1978 to 2012. These include 22 published articles, 1 unpublished study, 8 proceedings from the Iranian conference of parasitology, and 4 graduate student dissertations, resulting in 52,294 individuals and 23,385 IgG seropositive cases. The random errors method was used for this meta-analysis. The result shows that the overall seroprevalence rate of toxoplasmosis among the general population in Iran was 39.3% (95% CI = 33.0%-45.7%). There was no significant difference in the seroprevalence rate between male and female patients. A significant linear trend of increasing overall prevalence by age was noted (P < 0.0001). In addition, the data indicates that there are high seroprevalence in groups who have direct contact with cats, consume uncooked meat and raw fruits or vegetables, in farmers and housewife, individuals who have a low level of education, and live in rural areas. To the best of our knowledge, this is the first systematic review of T. gondii infection seroprevalence in Iran, which shows a high prevalence of Toxoplasma infection (more than one third). We highly recommend further study for the purposes of aiding patient management and developing more efficient diagnostic tests and effective prevention approaches.

Keywords: toxoplasmosis, *Toxoplasma gondii*, Iran, general population, systematic review, meta-analysis





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# RESPONSE OF PLASMA CELLS TO TOXOPLASMA GONDII AMONG BLOOD DONORS IN IRANIAN POPULATION

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Toxoplasma gondii is a protozoan parasite which can be transmitted to human through a variety of routes including blood transfusion. This cross sectional study aimed to evaluate the seroprevalence of Toxoplasma infection and related epidemiological features among healthy blood donors.A total of 375 blood products in the Blood Transfusion Institute, Zahedan, Iran were tested for specific T. gondii antibodies (IgG and IgM) by ELISA method. Positive IgG anti-T. gondii samples were further tested for IgM anti-T. gondii antibody. A positive IgG test with negative and positive IgM test was interpreted as a chronic and acute toxoplasmosis, respectively. 94 (25%) of 375 blood donors had IgG anti-T. gondii antibodies. No one of them were positive for IgM anti-T. gondii antibodies. The 25-36 year age group showed a significant T. gondii infection (p = 0.02). Other characteristics of blood donors including male gender, blood group ABO or blood transfusion did not show an association with infection.Our results highlighted that asymptomatic blood donors, especially those with active parasitemia, may constitute a significant risk of transmitting toxoplasmosis to susceptible recipients.

Keywords: blood donors, plasma cell, *Toxoplasma gondii*, IgG, IgM, antibody

# SEROLOGICAL STUDY OF TOXOPLASMOSIS AMONG PREGNANT WOMEN REFERRING TO REFERENCE LABORATORY OF NIKSHAHR FOR PREGNANCY PERIOD TESTS IN 2012

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Toxoplasmosis is one of the most prevalent zoonotic diseases worldwide which infect humans following the consumption of raw vegtables or half-cooked meat or through contamination with infected cat feces, as well as congenital route through the placenta to the fetus. In pregnant women, Toxoplasmosis is mostly without any symptoms and in warm climatic conditions is usually more common than cool climatic and mountainous regions. The serological techniques are most important methods for diagnosis. In this cross - sectional study 185 serum samples were collected from pregnant women referred to reference laboratory of Nikshahr in 2012 and IgG and IgM antibody levels against Toxoplasma in their sera were examined using ELISA method. Out of all samples, 10.3% were IgG positive and 0.55% were both IgG and IgM positive for Toxoplasmosis. Among participants in this survey 98.9% had the history of consuming raw vegetables, 9.7% of consuming half-cooked meat, 78.9% of not using disinfectant materials in washing vegetables, 5.4% had the history of contacting cats and 16.8% had consumed contaminated water. Given the low level of safety and risks of infection during pregnancy and its complications, promotion measures on public education and health care system should be carried out to prevent the infection.

Keywords: toxoplasmosis, serological techniques, Iran





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# SEROPREVALENCE OF TOXOPLASMOSIS IN DOMESTIC ANIMALS (SHEEP AND CATTLE) SLAUGHTERED IN KHORRAM ABAD, IRAN, 2010-2011

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Toxoplasmosis is a zoonotic disease caused by a protozoan parasite named Toxoplasma gondii. There is no clear information on the prevalence of this infection in cattle and sheep in Lorestan province. Therefore, in this study we aimed to determine the prevalence of Toxoplasma infection in domestic animals (sheep and cattle) slaughtered in khorram Abad. Totally, 446 blood samples were collected from sheeps and cattle slaughtered in Khoram Abad abattoirs. Sera were separated and tested for the presence of specific IgG anti-Toxoplasma gondii by an enzyme-linked immunosorbent assay (ELISA) technique. According to the guidelines of serologic kit manufacturer, 16.1% of sheeps and 11.9% of cattle were infected by Toxoplasma gondii. There was a significant relationship between seropositivity for Toxoplasma gondii and the age of animals (p=0.002). The infection rate was higher in older animals. The seroprevalence rate of infection was lower than the reported rates for many parts of the country. Actual infection rate may be slightly different from the value we determined, because serologic testing is not very accurate.

Keywords: *Toxoplasma*, toxoplasmosis, seroepidemiology, domestic animals

# CYST FORMATION FROM VIRULENT RH STRAIN OF TOXOPLASMA GONDII TACHYZO-ITE: IN VITRO CULTIVATION

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Some Toxoplasma gondii strains that produce tissue cyst in mice can spontaneously develop tachyzoite and bradyzoite in cell culture. We used the virulent RH strain which had never been shown to form cysts spontaneously in vitro. We aimed to induce conversion of RH strain tachyzoites to bradyzoites by changing the pH of the culture medium. HeLa cell monolayers were infected at a 1:1 tachyzoite to cell ratio. Four hours after infection, the culture medium was removed and replaced with medium culture and 5% FCS, adjusted to pH 8 with NaOH. The culture stored at 37°C in air to avoid pH variation due to CO2 until the end of the experiment. Cystlike structures were stained with Periodic Acid Schiff (PAS). The soluble antigens of T. gondii tachyzoite and bradyzoite of RH strain, bradyzoites of Tehran strain and uninfected HeLa cells were run on 12.5% Polyacrylamide gel and electrophoresed. The gel was stained by coomassie brilliant blue. Four days after infection of HeLa cells with RH strain of T. gondii tachyzoites, Cyst-like structures were noticed upon staining with PAS. In the SDS-PAGE, the protein bands were not quite similar to T. gondii tachyzoites and bradyzoites of RH strain but they were quite similar to T. gondii bradyzoites of RH and avirulent Tehran strains. P34 and P36 (bradyzoite-specific proteins) were observed only in T. gondii bradyzoites of RH and avirulent Tehran strains. This study showed, alkalization of culture medium to pH 8 induced expression of bradyzoite-specific proteins and production of RH cysts in cell culture.

Keywords: Toxoplasma gondii, RH strain, cell culture, cyst





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# Guilan, Iran; 20-22 May 2015 NEOSPORA CANINUM AND TOXOPLASMA IDEN GONDII INFECTIONS IN COW WITH HISTORY GONDI OF ABORTION IN HAMEDAN, IRAN GONDI

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Neospora caninum and Toxoplasma gondii cause abortion in cattle worldwide. The main aim of current investigation was to obtain seroprevalence of Neospora caninum and Toxoplasma gondii infections in aborted cattle in Hamedan province, west of Iran. A number of 85 blood samples were collected from aborted cattle of different regions of Hamedan province during the period 2010 to 2012. All samples were evaluated for IgG-antibodies to N. caninum and T. gondii using ELISA. 61.2%, 5.9% and 3.5% of samples were positive to N. caninum, T. gondii and co-infection, respectively. There was no significant correlation between seroprevalence rates, age groups and breeding (P>0.05). Also, a significant difference was found between T. gondii infection and the type of cattle (P=0.05), unlike to N. caninum (P=0.52).N. caninum and T. gondii are important causative agents for abortion in cattle in Hamedan province. Although the rate of T. gondii infection is low, this may partly be responsible to be transmitted to humans.

Keywords: Neospora caninum, Toxoplasma gondii, ELISA, abortion, cattle, Hamedan

# IDENTIFICATION OF LIVE TOXOPLASMA GONDII BY THE NASBA METHOD IN RATS

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Toxoplasmosis is a worldwide disease, for which different detection methods have been used. The nucleic acid sequence-based amplification (NASBA) method is proved to be highly efficient for diagnosis of live microorganisms. The present research evaluates the molecular isothermal method of NASBA to identify live Toxoplasma gondii (T. gondii) in rat. Tachyzoites of T. gondii were inoculated in the peritoneal cavities of mice (Mus musculus) and their RNA was extracted. The NASBA method was then used to amplify the tachyzoite B1 rRNA gene. Next, we examined blood samples from 30 experimentally infected case and control rats (Rattus norvegicus) by NASBA. Finally, the resultant band was investigated on an agarose gel. The B1 genes extracted from both the tachyzoites and blood samples were successfully amplified by the NASBA method. This amplified gene yielded an amplicon of approximately 116 bp on gel agarose.NASBA is highly efficient for the identification of live T. gondii. This method can be applied for early diagnosis of active toxoplasmosis in both newborns and immunocompromised individuals.

Keywords: Toxoplasma gondii, NASBA method, B1 rRNA gene





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# A MOLECULAR BEACON-BASED REAL TIME PCR ASSAY FOR QUANTITATIVE DETECTION OF TOXOPLASMA GONDII IN EXPERIMENTALY INFECTED RATS

#### <u>Roghayeh Norouzi</u><sup>1</sup>, Abdolhossein Dalimi Asl<sup>2</sup>, Mahdi Forouzandeh<sup>3</sup>, Fatemeh Ghaffarifar<sup>3</sup>

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Toxoplasmosis may cause significant damage to the developing fetus and is a life-threatening opportunistic infection in immunocompromised persons. Molecular methods have been known as more sensitive and more specific than serological test for diagnosis of toxoplasmosis. Application of quantitative real time PCR has evolved as a sensitive, specific, and rapid method for the detection of Toxoplasma gondii (T. gondii). The present study aimed to evaluate the efficacy of real time PCR method, using B1 gene, for the diagnosis of toxoplasmosis in the experimentally infected rats. Parasites were cultured in peritoneal cavity of mice and then the DNA was extracted in tachyzoite stage. The B1 gene of T. gondii was amplified by PCR and detected by real time PCR method based on the molecular beacon probe. Finally, real time PCR was evaluated for the quantization of T. gondii in the blood of the experimentally infected rats. The B1 gene of T. gondii which was successfully amplified by PCR yielded an amplicon with an approximate length of 116 bp. using this gene was evaluated highly appropriate for the quantization of T. gondii by real time PCR method.Application of real time PCR method is shown to be highly efficient in terms of sensitivity and rapidity for the detection of B1 gene as well as the quantization of T. gondii in blood of rat.

Keywords: *Toxoplasma gondii*, real time PCR, molecular beacon probe, B1 gene, rat

# COMPARISON OF TOXOPLASMA GONDII SERO-POSITIVITY IN HEMODIALYSIS AND PERITO-NEAL DIALYSIS PATIENTS IN TABRIZ

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Toxoplasmosis is the most common protozoal infection among immunocompromized individuals. It has been shown that prevalence of Toxoplasma gondii is high among Chronic Kidney Disease (CKD) patients. Due to lack of information about the seroprevalence of Toxoplasma in hemodialysis patients and peritoneal dialysis (PD) population, this study was an attempt to clarify Toxoplasma gondii seropositivity in a group of PD patients and compare it with the results of hemodialysis patients and seropositivity of general population in a highly prevalent area in Northwest Iran. The study was performed on 42 peritoneal dialysis patients, 84 hemodialysis patients, and 50 healthy volunteers. Anti-Toxoplasma IgG and IgM Ab tests were performed on the collected sera using ELISA method. Data was analyzed usig STATA 11 software and Chi- square. 70.2% of hemodialysis patients, 66.6 % of peritoneal dialysis patients and 68% of Control volunteers were positive for Anti- Toxoplasma IgG antibodies. All subjects were found as negative for IgM. There was no difference between Toxoplasma gondii seropositivity in hemodialysis patients and peritoneal dialysis patients and with general population (P > 0.05). None of the following risk factors including the history of indoor contact with cat, HBsAg and anti-hepatitis C virus antibody positivity and dialysis duration has any meaningful effect on the rate of Toxoplasma seropositivity in our studied cases except for diabetes mellitus.We concluded that either peritoneal dialysis or hemodialysis does not increase the risk of Toxoplasma seropositivity in our region.

Keywords: Toxoplasma gondii, hemodialysis, peritoneal dialysis, seropositivity, Iran





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THE COMPARISON OF ANTI-TOXOPLASMOSISIS ANTIBODY (IGM, IGG) IN HEMODIALYSED PA-TIENTS AND THOSE UNDERGOING CHEMOT-ERAPY WITH HEALTY BLOOD DONOR, SHAHR-E-KORD, 1392

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Toxoplasmosis as a zoonosis disease can infect human in different ways. Seroepidemiological studies show that infection with toxoplasmosis is prevalent worldwide and in some regions up to 70% of people have anti-toxoplasmosis antibody in their sera. Consumption of food contaminated with cat feces, contact with cat feces, eating raw or undercooked meat and parasite transfer from mother to child are major routes of human infection. Due to different ways of infection, rate of infection varies in different social groups. This study aimed to compare anti-toxoplasmosis antibody (IgG, IgM) among hemodialysis subjects undergoing chemotherapy in Hajar hospital and apparently healthy blood donors. In this comparative study, 62 hemodialysed patients and 27 subjects undergoing chemotherapy (89 overall) as the case group and 100 healthy blood donors as the control group were screened for anti-toxoplasmosis antibody (IgG, IgM). For this purpose, sera of all participants were separated and stored at -20°C until laboratory work initiated. Then samples were studied in order to compare anti-toxoplasmosis antibody (IgG, IgM) between two groups using ELISA and commercial kit (A-prodiagnostic). Data were analyzed using SPSS version 15 (applying statistical tests ANOVA and Chi 2). The mean age of all participants was 44.41± 3.8 years. According to age groups, there was no statistically significant difference between the case and control groups. Out of 62 hemodialysed patients and 27 cases receiving chemotherapy drugs, 28 cases (45%) and 9 cases (33.3%) had anti-toxoplasmosis antibody (IgG) in their sera, respectively. The rate of antitoxoplasmosis antibody (IgG) presence in the serum of healthy blood donors was 33% which was similar to that of the chemotherapy group. No statistically significant difference was found in terms of anti-toxoplasmosis antibody (IgG) between the case and control group. There was no statistically significant difference between case and control in terms of toxoplasmosis infection. Also, no relationship was found between presence of anti-toxoplasmosis antibody (IgG, IgM) and subjects' job, place of living, type of disease and duration of treatment. However rate of toxoplasmosis infection was statistically higher in  $\geq$ 55 years olds compared to other age group.

Keywords: toxoplasmosis, blood donors, hemodialysis, chemotherapy

THE CORRELATION BETWEEN TOXOPLASMA GONDII INFECTION AND PARKINSON'S DIS-EASE: A CASE-CONTROL STUDY

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Introduction and Objectives: Toxoplasma gondii as a worldwide zoonotic parasite can infect human by consuming tissue cyst using raw or undercooked meat, mature oocyte in contaminated food and water or vertical transmission from mother to fetus. Parkinson's disease (PD) as neurodegenerative disease infects people above 60 years. Due to high prevalence of toxoplasmosis in Iran and evidence about effects of Toxoplasma gondii on neurodegenerative diseases, this study has been conducted to investigate probable relationship between Toxoplasma gondii infection and Parkinson's disease in Iran. Seventy five Parkinson's patients and 75 healthy volunteers were enrolled. After obtaining informed contest and sociodemographic features, 5 ml blood sample were collected and Anti-Toxoplasma IgG and IgM tests were performed on sera by ELISA method. Data was analyzed usig STATA 11 software. Binary logistic regression was used for multivariate analysis in assessing the relationship between toxoplasmosis and Parkinson's disease. 69.3% of Parkinson group and 62.6% of control group were positive for Anti- Toxoplasma IgG antibody. All subjects were found as negative for IgM. No statistical differences between groups and age, gender, residency, consumption of raw or undercooked meat was observed. However, statistically significant association was found to exist between PD and cat ownership (P=0.01) as well as the consumption of undercooked egg (P=0.002). A significant association between IgG positive titer and Parkinson disease was not observed.while there was high Anti Toxoplasma IgG Antibody in Parkinson patients, we didn't find any association between Toxoplasma gondii infection and Parkinson disease.

Keywords: Toxoplasma gondii, Parkinson's disease, ELISA





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# STUDY OF IGG AND IGM ANTI-TOXOPLASMA GONDII ANTIBODIES IN SERA OF DIABETIC PREGNANT WOMEN IN AHVAZ COUNTY

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Toxoplasmosis is a parasitic infection caused by Toxoplasma gondii. Primary maternal infection with toxoplasmosis during pregnancy is frequently associated with transplacental transmission to the fetus and can lead to hazardous consequences such as abortion, different degrees of mental or physical retardation, epilepsy, blindness and death of the fetus by intensive infections. Studies showed that diabetic patients are suffering from weak immunity and one of the important groups among diabetic individuals, are diabetic pregnant women. Early diagnosis of acute toxoplasmosis in pregnant women and treatment of the infection can prevent damages. The aim of this study was to determine the IgG and IgM anti- Toxoplasma gondii antibodies in sera of diabetic pregnant women in Ahvaz County. In this analytic descriptive study, sera of 110 diabetic pregnant women as well as 110 sera of healthy pregnant women referred to the teaching hospital of Ahvaz Jundishapur University of Medical Sciences were collected and studied by ELISA and IFA methods to determine the IgG and IgM anti- Toxoplasma gondii antibodies. By ELISA method, the prevalence rates of IgG and IgM antibodies in diabetic pregnant women were 47 (42.9%) and 3 (2.9%) respectively and in healthy pregnant women was 24 (21.81%) for IgG antibodies. No IgM antibody was detected in control group. By IFA method the prevalence rates of IgG and IgM antibodies in diabetic pregnant women were 46 (41.8%) and 3 (2.9%), respectively and in healthy pregnant women the rate of IgG was 21 (19.09%). With this method no IgM antibody was detected in control group. In two ELISA and IFA methods, the obtained results for case group were statistically more significant than that of control group (p<0.05). The IgG and IgM anti- Toxoplasma gondii antibodies in diabetic pregnant women have higher prevalence rates in comparison with healthy pregnant women. It seems that screening tests for determination of the patients and teaching about transmission paths of Toxoplasma gondii should be considered in diabetic pregnant women surveillances.

Keywords: IgG, IgM, antibody, *Toxoplasma gondii*, diabetic patients, pregnancy

# A SEROLOGICAL STUDY ON CHOLESTEROL, HDL AND LDL CHANGES IN PATIENTS DUE TO TOXOPLASMOSIS IN ARDABIL, IRAN

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Toxoplasmosis is an important infection caused by Toxoplasma gondii. This protozoal infection is prevalent among many warm-blooded animals and spreads worldwide. Toxoplasmosis could lead to still birth in pregnant women and death in immunodeficiency cases. The purposes of this study were determination of three important lipids of serum Cholesterol, HDL and LDL of individuals infected with toxoplasmosis. In this descriptive and analytical cross sectional study, 200 subjects referred to Farabi laboratory (Ardabil) during summer and fall 2014, were examined. After preparation of blood samples and sera, electro chemiluminescence (ECL) technique was used to detect contamination of toxoplasmosis and for determination of the serum levels of cholesterol, HDL and LDL lipids, autoanalyzer technique was applied. Finally data was analyzed through SPSS19 using independent t-test method. From 200 studied cases (150 women and 50 men), 22 cases (14 wemen and 8 men) were positive for toxoplasmosis (Anti-toxoplasmosis IgG were above 30). Cholesterol, HDL and LDL lipids were measured in both case (22 infected cases) and control (22 non-infected persons) groups. The mean of Cholesterol, HDL and LDL were 147.27, 32.23, 95.63 in control group and 173.59, 49.32 and 94.91 in case group respectively. The amount of cholesterol and HDL in case group (Toxoplasmosis positive) were higher than control group (cholesterol Sig.= 0.016 and HDL Sig.= 0.001), whereas the amount of LDL showed no significant difference between these two groups (LDL Sig.= 0.92).

Keywords: cholesterol, HDL, LDL, toxoplasmosis, Ardabil





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INVESTIGATION OF ANTI-TOXOPLASMA GONDII ANTIBODIES IN THALASEAMIA MAJOR PATIENTS IN AHVAZ, IRAN

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Toxoplasma gondii is an intracellular parasite which can be transmitted through blood and blood products. Blood transmitted parasite can infect patients in treatment process of thalassemia. This study aimed to assess the prevalence of IgG and IgM antibodies in patients with thalassemia. In this cross-sectional study from March 2012 to November 2013, blood samples were collected from 100 patients with thalassemia and 100 healthy individuals and separated serum antibodies IgG and IgM anti-Toxoplasma in samples measured by ELISA. Demographic data were collected from two groups. The data enterred in SPSS software and analyzed using chisquare test. The prevalence of IgG antibodies in patients with thalassemia, 28 (28%) was significantly higher than the control group, 11 patients (11%) was significantly higher (p <0.001). The prevalence of IgM antibodies in thalassemia patients (2%) was significantly higher (p <0.002) than the control group (0%). Toxoplasma prevalence in males was higher than females.Because in major thalasemia, immune responses decrease in long term and possibility of bloodborne diseases raise in transfusion, further follow-up is required. In this study statistically significant difference was seen between test and control groups, and also blood donor should be checked for infectious agents such as Toxoplasma gondii.

Keywords: Toxoplasma gondii, thalasemia, antibody

FREQUENCY DISTRIBUTION OF BLOOD-TISSUE PROTOZOA INFECTIONS IN PATIENTS WITH MULTIPLE SCLEROSIS (MS), AS COMPARED TO THE CONTROL GROUP, ISFAHAN, IRAN, 2012

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A general look at the status of health clearly reveals a considerable advance in control of infectious diseases in modern societies in comparison to the past, especially in control of water, food and insect- borne diseases. On the other hand, diseases that are recognized as autoimmune are increasing. In this article, efforts have been made to investigate the frequency distribution of blood tissue parasitic infections in patients with multiple sclerosis (MS), in comparison to a control group in Isfahan province in 2012, and also to take another look at the interaction and the link between these diseases. Parasites studied included those capable of long-term stimulation of the immune system, causing chronic diseases. This was specifically an epidemiological study in which case groups consisted of 50 patients with multiple sclerosis and control group of 50 family members of these patients (for consistency in socio-economic status and genetic factor). Serum samples were analyzed for anti-Toxoplasma gondii IgG and IgM antibodies using a commercially available ELISA kit, and the data was analyzed using SPSS. Of the study population, 36% in case group and 49% in the control group had positive test in IgG and IgM Toxoplasma gondii were negative but the difference between the two groups was not significant. In terms of other infections (cutaneous Leishmaniasis, and malaria), there was no significant difference between the two groups, either. Health education towards avoiding eating undercooked and raw meat or milk, and avoiding contact with cats were recommended, especially during pregnancy.

**Keywords:** multiple sclerosis, cutaneous leishmaniasis, malaria, toxpolasmosis, IgG, IgM, Isfahan, Iran





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# PREVALENCE OF TOXOPLASMA GONDII ANTI-BODY AND OOCYST SHEDDING IN STRAY CATS OF KHORRAMABAD, WEST OF IRAN

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Toxoplasma gondii is a worldwide parasite infecting the central nervous system of warm-blooded animals, including humans. The infection is acquired mainly by eating food or water contaminated with oocyst excreted by cats, or tissue cysts of T. gondii in the under-cooked meat. The most common clinical form of human toxoplasmosis is lymphadenitis but the major clinical problem of toxoplasmosis is congenital infection of fetus, resulted from primary infection during pregnancy, as well as ocular toxoplasmosis and reactivated form in immunocompromised patients. T. gondii is a main cause of abortion in the TORCH syndrome and therefore, one of the most important infection agents causing the abortion and congenital abnormalities in the human. The aim of this study was determining the prevalence of T. gondii antibodies and oocyst shedding in a population of stray cats in Khorramabad, west of Iran. A total of 125 stray cats trapped from different parts of city and were brought to the research laboratory for taking blood and faces specimens. The blood samples of the cats (71 males and 54 females) were assayed for the prevalence of T. gondii using the IgG-ELISA, and their fresh fecal samples collected and sugar floatation concentration method was applied for detection of oocyst. From 125 cats, 71 (56.8%) were male. T. gondii -like oocysts were detected in only 3 of 125 samples tested from cats' stool by direct microscopy and floatation methods (the oocyst size was out of the range). Antibodies were found in 80 out of 125 cats (64%). The prevalence of sero-positive male cats (69%) was higher than female (57.4%) cats but the difference was not significant. There was no significant difference in the T. gondii antibody titers between males and females, or between cats living in different parts of city, but prevalence rate between different age groups were significant statistically (P=0.021). A significant proportion of cats from Khorramabad have been exposed to Toxoplasma. The result of this study may have implications for the human health promotion, especially for pregnant women.

Keywords: Toxoplasma gondii, prevalence, oocyst, ELISA, cat

# SEROLOGICAL STUDY OF NEOSPORA CANINUM AND TOXOPLASMA GONDII INFECTION RATES IN DOGS IN HAMEDAN PROVINCE, IRAN

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The aim of this study was to determine the seroprevalence of *Neospora caninum* and *Toxoplasma gondii* infection rates in dogs from Hamedan province. Blood samples were collected randomly from 270 dogs (70 stray dogs and 200 shepherd dogs) in this area. All of samples were evaluated for antibodies to *N. caninum* and *T. gondii* using IFAT and Modified Agglutination Test (MAT), respectively. Antibodies to *N. caninum* and *T. gondii* user evaluated for cases respectively. Co-infection was not seen. Both infection rates in stray dogs were higher than shepherd dogs. There was statistical significant differences among age groups and type of dogs, opposite to gender (P=0.112). This study is the first report of *N. caninum* and *T. gondii* infection rate in dogs from west of Iran. The presence of infection in stray dogs should be considered as a risk factor for *N. caninum* infection in cattle.

Keywords: Neospora caninum, Toxoplasma gondii, dog, Hamedan





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SEROLOGICAL STUDY OF TOXOPLASMA GONDII INFECTION IN CATTLE FROM HAME-DAN PROVINCE, IRAN

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Toxoplasma gondii is an important zoonosis causes abortion in cattle worldwide. The aim of this study was to determine the seroprevalence of T. gondii infection in cattle from Hamedan province. 1,406 sera samples were evaluated for IgGantibodies to T. gondii in rural and industrial cattle from this area using ELISA. Thirty-two (2.3%) of samples were reported seropositive. The association between seroprevalence and rat control in farm (P=0.001), cat contact with herd (P=0.037), and age groups (P=0.002) was statistically significant. Also, seropositive rate in semi-intensive breeding system (rural farms) was higher than intensive system (industrial farms) (P=0.0001). There was not statistical differences between type of cattle (P=0.559), breeds (P=0.366), gender (P=0.316), and presence of abortion history (P=0.231). The current investigation is the first report of T. gondii infection in cattle from western Iran. Although the rate of infection is low, the results indicate that T. gondii infection may partly be responsible for transmission to humans and economic losses in cattle husbandry in this region.

Keywords: Toxoplasma gondii, cattle, ELISA, Hamedan, Iran

EVALUATION OF IMMUNIZATION WITH PLAS-MID ENCODING GRA4 ANTIGEN OF TOXO-PLASMA GONDII AND ALUM (ADJUVANT) IN BALB/C MICE

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Toxoplasma gondii is a widespread intracellular protozoan parasite responsible for animal and human toxoplasmosis. In immunodeficient patients, chronic infection with T. gondii can reactivate and produce encephalitis, which is often lethal. Vaccine strategy remains a promising method for the prevention and control of toxoplasmosis. GRA4 is a dense granule protein of Toxoplasma gondii has been identified as a potential candidate for vaccine development. In our study, we evaluated the immune response induced by five different immunization vaccination strategies encoding pcGRA4. The pGRA4 was transfected into E. coli (TG1) cells, and expression of GRA4 antigen was investigated by Western blotting and SDS-PAGE. Western blotting analyses of CHO cells transfected with pGRA4 showed specific expression of GRA4. Immunogenicity of pGRA4 was evaluated in mice. Balb/c mice were intramuscularly (I.M) immunized three times according to specific immunization schedules. Generally, Five group (PBC, PcDNA3, PcGRA4+ Alum, Alum) of BAIB/c mice (n=10 in each group) were selected. Mice in experigroups were mental immunized with pcGRA4, pcGRA4+Alum, Alum (Alum as an adjuvant) and mice in the control groups were injected with PBS, pcDNA3. After immunization, the levels of total IgG antibody and cytokine productions were determined by enzyme-linked immunosorbent assays (ELISA). The survival rate of mice was also evaluated after challenge with a lethal dose of T. gondii RH strain tachyzoites. The results showed that mice vaccinated with different immunization regimens (pcGRA4,pcGRA4+Alum ,Alum, pcDNA3, PBS) elicited specific humoral and cellular responses, with high levels of total IgG and gamma interferon (IFN-y),and low level of IL4 which suggested a specific Th1 immunity was activated. After lethal challenge, an increased survival rate was observed in immunized mice (11 days) compared to the control groups injected with PBS and pcDNA3 (6 days) (P < 0.05).Immunization with pc-GRA4 in alum induces partial protective immunity against lethal infection with T. gondii in mice, so pcGRA4+Alum can be a good candidate for anti-T. gondii vaccine.

**Keywords:** *Toxoplasma gondii*, pcGRA4 + alum adjuant, pcDNA3 plasmid, humoral Immunity, cellular Immunity.





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DETECTION OF ANTI- TOXOPLASMA GONDII IGG AND IGM ANTIBODIES IN CORD BLOOD SERUM SAMPLES BY ELISA METHOD

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Toxoplasma gondii is one of the most prevalent parasites of human and other warm blooded animals. Diagnosis of acquired infection is very important in pregnant women. The frequency of congenital toxoplasmosis in the world is around 5 per 1000. The purpose of this study was to estimate the prevalence of IgG and IgM antibodies in cord blood of newborns from Shahid Mostafa Khomeini Hospital, Tehran, Iran. A total of 1000 cord blood serum samples were collected during 1391-92. Anti T. gondii IgG and IgM antibodies were analyzed by ELISA method using home-made antigen. From1000 cord blood serum samples 198 cases (19.8%) were positive for anti T. gondii IgG antibody. Anti T. gondii IgG positive samples were examined for IgM antibody in which 2 samples showed upper OD from negative samples. These 2 samples need more complementary tests so the results will be declared subsequently. Detection of congenital T. gondii infection is very important for further measurements.

Keywords: *Toxoplasma gondii*, ELISA, cord blood, IgG antibody, IgM antibody

SEROEPIDEMIOLOGICAL STUDY OF TOXO-PLASMOSIS IN WOMEN REFERRED TO ARAK MARRIAGE CONSULTING CENTER DURING 2012-2013

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Toxoplasmosis is a zoonotic and usually appears as asymptomatic infection. Transmission of the parasite in the first trimester of pregnancy results in abortion or congenital abnormalities (convulsion, chorioretinitis and mental retardation in newborns). This study carried out to investigate seroepidemiology of Toxoplasma infection among women at risk of abortion, premature delivery, infant at risk of toxoplasmosis and affecting factors. In this cross-sectional and descriptive study serum samples were collected of 400 women referred to marriage consulting center in Arak city during 2012-2013. Anti-Toxoplasma IgG and IgM antibodies were measured by ELISA using home-made antigen. Finally results were analyzed by SPSS software. Chi-square test was used for comparison. From 400 tested sera of women referred to marriage consulting center in Arak city, 97(24.3%) had IgG antibodies against T. gondii and 19 cases (4.8%) were positive for IgM antibodies. A significant correlation was seen between T. gondii infection with clinical symptoms and keeping cat as pet animal, education, handling or eating raw or undercooked meat and immunodeficiency (p < 0.05). Rather low prevalence of Toxoplasma infection was found in Arak city (24.3%) compared to the results obtained from other cities of Iran. It seems that keeping cat as pet and consumption of undercooked liver and uncooked hamburger are the most important transmission routes of the infection. Since majority of Toxoplasma uninfected women (75.7%) live in Arak city, performing serological tests for screening and health education prior to marriage or during pregnancy are recommended for reducing the risk of congenital toxoplasmosis in this area.

Keywords: *Toxoplasma* infection, marriage, antibody, ELISA, Iran





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THE IN VITRO EFFECTS OF NANOSILVER COL-

### LOID (NANOSID) ON TOXOPLASMA GONDII TACHYZOITES

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Silver was the most important antimicrobial agent available before the introduction of antibiotics. It has been effective against almost all organisms tested and has been used for treatment of numerous infections. The present study was designed to investigate the possible anti *Toxoplasma gondii* efficacy of nanosilver colloid. Materials & Methods : *Toxoplasma gondii* tachyzoites were incubated with nanosilver colloid (5ppm,10 ppm,20 ppm) as test and with distilled water as control for 30, 60, 120 min. Then the activity and mortality rate of tachyzoites were degenerated in less than 30 minutes in comparison with control group. The experiment indicates that nanosilver can be used as an alternative disinfectant against *Toxoplasma gondii* tachyzoite.

Keywords: Toxoplasma gondii, tachyzoite, nanosilver colloid.

# STUDY OF TOXOPLASMA GONDII INFECTION IN FREE-RANGED, CAGED CHICKENS AND TURKEY IN HAMADAN, WEST OF IRAN, 2013

# Masoud Hamzekhani<sup>1</sup>, Amirhossein Maghsood<sup>2</sup>, Mohammad Matini<sup>2</sup>, Mehrdad Hajilooei<sup>2</sup>, <u>Mohammad Fal-</u> <u>lah<sup>3</sup></u>

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Toxoplasmosis is a widely prevalent zoonotic disease, caused by Toxoplasma gondii. Free-range chickens are considered as one of the most important hosts in the epidemiology of T. gondii infection because they are an efficient source of infection for both cats and humans. T. gondii-infected poultry (including chickens) is consumed widely in many countries, including Iran, and could be the primary source of infection for humans. There was no information regarding T. gondii infection in chickens in this area of Iran, therefore, this investigation carried out to determine the prevalence of T. gondii infections in poultry in Hamadan. A total of 203 birds including 162 free-ranged, 41 caged commercial chickens and 2 turkeys were studied from Hamadan city and suburb in the west of Iran, from January to July 2014. Blood samples were collected by venipuncture and transported to Research Laboratory of Department of Parasitology. Data regarding these birds (kind, age, gender and location) were also recorded. The head of birds also used for testing brain tissue regarding to T. gondii tissue cyst. Brain of each bird grinded and suspension were made by normal saline and inoculated to peritoneal cavity of 5 mice. Peritoneal aspirate was examined for tachyzoites of T. gondii after 5-10 days. The serums tested by indirect hemagglutination test (IHA) for T. gondii antibody. Tissue cyst of T. gondii was detected by bioassay in the brain of 3 out of the 203 associated samples (one FR and one caged) by peritoneal inoculation (1%). Seropositivity for T. gondii antibody was 6.1% (12/196). Positive cases were as follow: 6 FR hens, 1 caged chicken and 5 rooster. No positive cases were found in the turkeys. This study indicates that, both freeranged and caged chickens may have similar risk of infection to T. gondii and can transmit the parasite to humans.

Keywords: *Toxoplasma gondii*, prevalence, brain, IHA, mice, chicken, turkey





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# ANTI-TOXOPLASMA IGM AND IGG SEROPOSI-TIVITY IN ISFAHAN, CENTRAL IRAN

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Toxoplasma gondii is a protozoan parasite with wide variety of intermediate hosts, including humans. Human toxoplasmosis is mostly benign infection with negligible symptoms, but it can cause severe infection in immunocompromised individuals and infants during pregnancy. This study conducted in order to determine the prevalence of toxoplasmosis and some risk factors among humans referred to Dr. Sharifi Clinical Laboratory, Isfahan, Iran. Materials & Method: In a retrospective study, 1287 patients that had anti-Toxoplasma IgM or IgG test from January 2013 to January 2015 were selected and included in the study. Quantitative determination of anti-Toxoplasma IgM and IgG was performed using antibody capture chemiluminescence immunoassay (CLIA) kits (LIAI-SON® Toxo IgM and IgG, DiaSorin S.P.A, Italy) applied in LIAISON (DiaSorin, Germany) device. All the available variables such as anti-Toxoplasma IgG and IgM concentrations, sex and age were recorded and analyzed. Totally 1287 humans including 1215 (94.4%) females and 72 (5.6%) males with mean age of 28.64 year (min: 1, max: 78) were studied. The results showed that 36 (2.8%) out of 888 and 325 (25.3%) out of 1243 were anti-Toxoplasma IgM and IgG seropositive, respectively. The mean age observed significantly higher in IgG positive patients (P <0.001), but not in IgM positive patients (P= 0.065). No statistically significant relationship was observed in the IgM and IgG seropositivity and concentrations among sexes. According to the results of the present study, prevalence of toxoplasmosis is high in Isfahan area, but it is still lower than most of the other studied regions of the country. Also, the risk of the infection rises with increasing the age.

Keywords: Toxoplasma, IgG, IgM, Iran

# LETHAL EFFECTS OF PEGANUM HARMALAE AND ZIZIPHORA TENUIOR EXTRACTS ON TACHYZOITES OF TOXOPLASMA GONDII IN VITRO

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Synthetic anti-Toxoplasma drugs have different side effects, especially suppression of haematopoiesis that has a great importance in primary Toxoplasma infections during pregnancy. Access to a new drug with fewer side effects is a major objective of Toxoplasma researches. It has been shown that some of herbal extracts/fractions have inhibitory effects on tachyzoites of T. gondii in cell culture. In the present study, we have investigated lethal effects of Peganum harmalae and Ziziphora tenuior extracts on RH strain tachyzoites of T. gondii in vitro. Propagated tachyzoites in peritoneum cavity of mice were harvested and washed with saline. Afterward, 106 of fresh tachyzoites were treated with concentrations of 10, 50, 100, and 200 mg/ml of the extracts in microtubes, separately. After 10, 30, and 45 minutes incubation in room temperature, the tachyzoites were stained with alkaline methylene blue and then, percentages of killed tachyzoites were determined. All experiments were performed in triplicate with three replicates per sample. Also, bioassay in mice was used for conforming mortality of tachyzoites. The data were analyzed with One-Way ANOVA, Kruskal-Wallis, and Kolmogrov-smirnov tests by SPSS. The mortality rates of the tachyzoites were 100% with concentrations of 100 and 200 mg/ml of Peganum harmalae and Ziziphora tenuior extracts after 10, 30, and 45 minutes incubation in room temperature. In concentrations of 50 mg/ml of Peganum harmalae and Ziziphora tenuior extracts, the mortality rates of the tachyzoites were 94.24±1.72% and 6.32±4.7% after 45 minutes incubation, respectively. Also, in concentrations of 10 mg/ml of Peganum harmalae and Ziziphora tenuior extracts, the mortality rates of the tachyzoites were 13.75±12.12% and 5.82±2.01 % after 45 minutes incubation, respectively. By bioassay in mice, mortality of tachyzoites was confirmed.Based on our finding, Peganum harmalae and Ziziphora tenuior extracts have anti-Toxoplama activity with dose-dependent lethality.

Keywords: Toxoplasma gondii, herbal extract, Peganum harmalae, Ziziphora tenuior, in vitro





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# DETECTION OF TOXOPLASMA GONDII OO-CYSTS IN CAT FECES BY PCR SCREENING METHOD, IN AHVAZ

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Toxoplasmosis is a significant zoonosis infection that is caused by Toxoplasma gondii, a coccidian parasite of phylum Apicomplexa. This obligate intracellular parasite can infect 30% to 40% of humans as well as other warm blooded animals worldwide. Felids as definitive hosts in the life cycle of T. gondii have important roles in environmental infection. Contaminated soil with oocysts is a main source of infection for humans in park and gardening. For investigation of oocysts in cat feces, this study was performed for the first time in Ahvaz. 486 samples of cat feces were collected from different parts of Ahvaz, the capital city of Khuzestan province, Southwest of Iran. In order to recover the oocysts, samples were concentrated by using sodium nitrate flotation method. For specific detection, T. gondii DNA was extracted and PCR was performed targeting a 194 bp fragment of B1 gene. Presence of T. gondii DNA were successfully detected in 35 of 486 (7.2%) samples which were examined by PCR amplification.Abundance of stray cats in the environment and their role as potential source for transmission of T. gondii oocysts is very important. The results showed that PCR method based on B1 gene may have important role in detection of this infection.

Keywords: Toxoplasma gondii, cat, PCR, Ahvaz

# SEROEPIDEMIOLOGICAL STUDY OF TOXO-PLASMA GONDII IN MULTIPLE SCLEROSIS

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Toxoplasmosis infection commonly share between humans and animals in the world. People may acquire the infection through eating oocysts or contaminated meat and the congenital form of the disease can be transmitted from mother to the fetus through the placenta, which may cause fetal death, central nervous system damages or eye complications. The human form of the disease is often asymptomatic and sometimes a feeling of general discomfort, and swelling of the lymph nodes is associated with chorioretinitis. In people with problems of immune system, malignancy and those who undergo organ transplants acute infection may be fatal. The purpose of this study was to determine the prevalence of anti-Toxoplasma gondii antibodies in serum of multiple sclerosis patients referred to Iran's MS society. In this study blood samples of 100 MS patients and 100 control subjects were collected and examined for anti-Toxoplasma antibodies (IgG, IgM) by ELISA method. The results showed that the overall prevalence of anti-Toxoplasma IgG was 38%, and the prevalence of Toxoplasma IgM antibodies was negative in all subjects. In the control group 21% were positive for IgG while IgM was negative in controls. Pearson correlation coefficient determined- with precision P <0.0001- significant association between antibodies to Toxoplasma and MS disease.Because of high prevalence, serum titers regularly should be measured in MS patients. Also, these people should receive anti-parasitic treatment.

Keywords: Toxoplasma gondii, ELISA, MS patients





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CAN TOXOPLASMA GONDII BE INTRODUCED AS A RISK FACTOR IN ETIOLOGY OF ALZEIMER DISEASE?

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Alzheimer's disease is a progressive neurological disease that affects population over 65 years. Some infectious agents have been known as risk factor in etiology of Alzheimer's disease. Toxoplasma gondii is an important infectious agent in neurodegenerative diseases. The aim of this study was investigation of possible effect of Toxoplasma gondii infection as a risk factor in etiology of Alzheimer's disease. This case-control study has been conducted on 75 Alzheimer's patients and 75 healthy volunteers. After obtaining informed contest and filing in questionnaire, 5 ml blood samples were obtained from patients and healthy volunteers. After separation of plasma, Anti-Toxoplasma IgG and IgM tests were done on them by ELISA technique. Data were analyzed using Chisquare and Fisher's test by STATA 11 software. In this study, 61.3% of Alzheimer's patients and 62.6% of healthy volunteers were positive in term of Anti-Toxoplasma IgG. All participants were negative for Anti-Toxoplasma IgM. After statistical analysis, there was no significant differences between Alzheimer's patients with their controls in term of Anti- Toxoplasma IgG Ab (P=0.5). This result shows that infection with Toxoplasma gondii cannot be regarded as a risk factor for developing Alzheimer's disease.

Keywords: Alzheimer's disease, *Toxoplasma gondii*, ELISA, Iran

# INVESTIGATION OF TOXOPLASMA GONDII GENOTYPES ISOLATED FROM SLAUGHTERED SHEEP IN JAHROM BY PCR-RFLP

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Phylogenic studies have showed that the strains of Toxoplasma gondii are divided to three genetic groups. Infection with this parasite can causes a different clinical manifestations in human and animals. Recent studies suggest that the genetics of the parasite can play a role in progression and severity of disease. Since, the consumption of raw or undercooked meat of livestock has been regarded as a major source of infection for humans, this study is conducted to determine the genotypes of T. gondii from slaughtered sheep in Jahrome. This cross sectional descriptive study was carried out on the samples from slaughtered sheep in Jahrom during 2012-2103. After DNA extraction with phenol-chloroform method, genotyping was determined by Nested PCR- RFLP based on restriction pattern of 3' and 5' ends of SAG2 gene. RFLP analysis for 125 samples were positive for Toxoplasma infection, showing that there were 83.2% type I, 3.2% type II, 11.2% type III and 2.4% mix genotypes II and III. A significant relationship between genotypes and sheep gender was found but not between genotypes and sheep age. This study showed that the most frequent genotypes of Toxoplasma in sheep are genotype I with highest virulence and pathogenicity. Therefore, freezing and complete cooking of meat is a necessary attempt for prevention of toxoplasmosis in the area.

Keywords: Toxoplasma gondii, genotype, sheep, Nested PCR-RFLP





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LETHAL EFFECTS OF CARUM COPTICUM, HY-PERICUM PERFRATUM, ACHILLEA MILLEFOLI-UM EXTRACTS ON TACHYZOITES OF TOXO-PLASMA GONDII IN VITRO

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Manufacturing anti-Toxoplasma drugs with fewer side effects is a research priority. Synthetic drugs of toxoplasmosis were associated with restrictions. Studies in recent years have shown that some herbal extracts/fractions have significant anti-Toxoplasma effects. In this study, the cytotoxic effects of ethanol extracts of Carum copticum, Hypericum perfratum and Achillea millefolium on T. gondii RH strain tachyzoites were evaluated in vitro. The RH strain of T. gondii tachyzoites were proliferated intraperitoneally in mice. The tachyzoites were collected, washed and counted. Then, 50 ml suspension contains 106 tachyzoites were exposed to 50 ml of each extract at concentrations of 10, 50, 100, 200 mg/ml within 10, 30 and 45 minutes at the laboratory temperature. Afterward, the tachyzoites were stained with alkaline blue methylene. Experiments were repeated three times, triple. Bioassay in mice was applied to confirm mortality of tachyzoites. The data were analyzed with One-Way ANOVA, Kruskal Wallis, and Kolmogrov-smirnov tests. At concentrations of 50, 100 and 200 mg/ml of Hypericum perforatum extract, 100% tachyzoites in each incubation time of 10, 30 and 45 min were killed. At concentration of 10 mg/ml of this extract, the percentage of killed tachyzoites after 45 min of incubation was 5.3  $\pm 1.5\%$ . Extract of Achillea millefolium at concentration of 100 and 200 mg/ml could kill 100% of tachyzoites within 10, 30 and 45 min of incubation time. The mortality percents of Achillea millefolium extract at concentrations of 50 and 10 mg/ml were 5.24  $\pm$  4.6% and 5.1  $\pm$  1.9% within 45 min. The mortality rates of Carum copticum extract at concentrations of 50 and 10 mg/ml were 15.77±7.00% and 5.3±3.47% within 45 min, respectively. Also, 100% mortality of tachyzoites were confirmed by bioassay. Extracts of Hypericum perforatum, Achillea millefolium and Carum copticum have anti-Toxoplama effects.

Keywords: Toxoplasma gondii, herbal extract, Carum copticum, Hypericum perfratum, Achillea millefolium, in vitro

# ANTI-TOXOPLASMA ACTIVITY OF ETHANOLIC EXTRACTS OF MYRTUS COMMUNIS, ARTEMISIA AUCHERI AND PIPER NIGRUM IN VITRO

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Synthetic anti- toxoplasmosis drugs have considerable side effects with no efficacy on bradyzoites enclosed in tissue cysts. In the recent years, limited studies have indicated that some herbal extracts have noticeable anti-Toxoplasma effects. In this study, the fatal effect of ethanolic extracts of Myrtus communis, Artemisia aucheri and Piper nigrum on tachyzoites of Toxoplasma in vitro was studied. RH strain tachyzoites of T. gondii were harvested after intraperitoneal propagation in mice. Then, 106 of tachyzoites in 50 µl/ml of saline were exposed to 50 µl of ethanolic extracts of Myrtus communis, Artemisia aucheri and Piper nigrum in concentration of 200, 100, 50 and 10 mg/ml in 10, 30 and 45 min in room temperature. After incubation, tachyzoites were stained with alkaline methylene blue. The mortality rates of tachyzoites were microscopically determined. All experiments were performed in triplicate with three replicates per sample. Bioassay in mice was used to confirm 100% mortality of tachyzoites treated with extracts. The data were analyzed with One-Way ANO-VA, Kruskal Wallis, and Kolmogrov-smirnov tests by SPSS software. The mortality rate of tachyzoites by extract of Myrtus communis at the concentrations of 200, 100, 50 mg/ml in 10 minutes incubation was 100 %, and decreased to 5.91  $\pm$ 2.6% at the concentration of 10 mg/ml. The mortality rates were 100, 97.6  $\pm$  2.3, 64.8  $\pm$  21.2, 9.0  $\pm$  4.3 % with concentrations of 200, 100, 50 and 10 mg/ml Artemisia aucheri extract in 10 min, respectively. The fatal effect of Piper nigrum with the same concentrations at 10 min was  $12.0 \pm 5.4$ ,  $10.0 \pm 3.7$ ,  $9.5 \pm 4.1$ ,  $6.5 \pm 3.9\%$ , respectively. Conclusions: The extracts of Myrtus communis and Artemisia aucheri have significant fatal effects on T. gondii.

**Keywords:** Toxoplasma gondii, herbal extracts, Myrtus communis, Artemisia aucheri, Piper nigrum





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#### AN INVESTIGATION ON BIOCHEMICAL CHANGES IN PATIENTS INFECTED WITH TOX-OPLASMOSIS IN RASHT, IRAN

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Toxoplasmosis is a worldwide prevalent infection that is caused by a protozoan called Toxoplasma gondii. The complications of this infection are abortion in pregnant women, chorioretinitis (blindness) in suffering newborns and mortality in patients with immune deficiency. The parasite could migrate to several organs such as eye, heart, brain, muscles and liver, localize there and construct tissue cysts. The purpose of this study was to assess the biochemical changes of alkaline phosphatase (ALP), urea, albumin and alanine aminotransferase (ALT) in toxoplasmic individuals. In this descriptive and cross-sectional study, we selected 80 patients infected with toxoplasmosis by measuring IgG, IgM (ELISA technique) from the people who referred to Sina laboratory in Rasht city (north of Iran) during summer and fall 2014. Then, we determined the levels of ALP, albumin, urea, and ALT (SGPT) in the sera of both case (80 infected patients) and control (100 non-infected individuals) groups. The results of this study indicated that the levels of albumin (5-6.2 g/dl), ALP (310-440 IU/L) and ALT or SGPT (40-55 IU/L) in the blood of acute toxoplasmosis infected individuals were higher than non-infected persons. There was no significant difference between the amount of urea in toxoplasmosis infected and non-infected groups.We concluded that the elevated levels of ALT (SGPT), ALP and albumin in the blood of toxoplasmosis infected patients could suggest liver damage caused by toxoplasmosis.

Keywords: toxoplasmosis, biochemical changes, Rasht, Iran

#### PHENOTYPING OF ABO BLOOD GROUPS IN THE PATIENTS INFECTED WITH TOXOPLAS-MOSIS IN RASHT, IRAN

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Toxoplasmosis is an important parasitic disease caused by Toxoplasma gondii. This protozoal infection is prevalent among many of warm-blooded animals and human. It could lead to abortion and still birth during first semester of pregnancy both in sheep and human. In the persons with acquired immune deficiency, such as AIDS, it could be lethal. This study was designed to determine the phenotype of ABO blood groups among toxoplasmosis infected individuals in Rasht (north of Iran). In this descriptive and cross-sectional study, we collected the blood samples of 180 individuals (with suspected symptoms of toxoplasmosis) who referred to Sina laboratory (Rasht) during summer and fall 2014. First we recognized toxoplasmosis infected cases using ELISA technique (by detecting of anti-Toxoplasma IgG and IgM). Then we determined the phenotype of ABO blood groups among the infected individuals, by tubular (back type) method. Of 180 studied samples, 80 cases were positive for toxoplasmosis infection. All of the patients belonged to 18-30 year-old group and 60 percent of them were women. 50 percent of the toxoplasmosis infected cases possessed O blood group, while 20% of them had AB blood group, 17.5 percent B and 12.5 percent A blood group.We concluded that toxoplasmosis infection among the people having O blood group is more prevalent than other blood groups, and the people with O blood group are more susceptible to the infection of toxoplasmosis.

Keywords: phenotype, ABO blood group, toxoplasmosis, Rasht, Iran





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BRAIN CYSTOGENESIS CAPACITY OF TOXO-

# PLASMA GONDII, AVIRULENT TEHRAN STRAIN

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Introduction & Objectives. The awareness on brain cystogenesis capacity of T. gondii strains will be helpful for studies of experimental infections by this parasite in mice. In this regard, this is the first report on Tehran strain of T. gondii isolated from a patient with lymphadenitis in 1973. Materials & Methods. A volume of 0.5 ml mice brain suspension containing 20 tissue cysts of Tehran strain of T. gondii was inoculated intraperitoneally to each of 25 male Balb/c mice. The number of brain cysts was counted in unstained crushed smears for ten mice during weeks 7-9 and for fifteen mice during weeks 13-14 post- infection (p.i.). Nonparametric test of Mann-Whitney was used to demonstrate means differences. Results. There was a significant difference in the means for the number of brain cysts during weeks 7-9 and weeks 13-14 p.i. (P<0.05), means (±S.E.M) of 228.3 ± 144.8 and 1239.8  $\pm$  429.3 for the number of brain cysts, respectively. The minimum and the maximum of cysts were 70 and 1531 during weeks 7-9 p.i., and 12 and 5170 during weeks 13-14 pi, respectively. The mean number of brain cysts in the right cerebral hemisphere was non-significantly higher than the left cerebral hemisphere. Furthermore, the number of cysts counted in the right or the left hemispheres was significantly higher than those enumerated for cerebellum + brain stem altogether. Conclusion. It was concluded that the brain cystogenesis capacity of T. gondii, Tehran strain shows enormous variation in mice regarding the duration of infection. In addition, the cystogenesis observed in cerebellum + brain stem was lower than the right and left cerebral hemispheres.

Keywords: Toxoplasma gondii, Tehran strain, brain cyst, mouse

#### RECOMBINANT EXPRESSION AND IMMUNO-LOGICAL ACTIVITIES OF DENSE GRANULE AN-TIGEN 7 OF TOXOPLASMA GONDII

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The precise diagnosis of the acute toxoplasmosis in pregnant women and immunocompromised patients has critical importance. Most of the commercially available assays use the whole Toxoplasma soluble extract as antigen. However, the assays currently available for the detection of specific anti-Toxoplasma antibodies may vary in their abilities to detect serum immunoglobulins, due to the lack of purified standardized antigen. The aim of this study was production and evaluation of the usefulness of the recombinant Toxoplasma gondii GRA7 antigen for serodiagnosis of Toxoplasma gondii by ELI-SA. DNA fragment encoding the GRA7 antigen of T. gondii was extracted from the total DNA of T. gondii RH strains by PCR. The gene easly cloned in pTZ57R/T. The gene with correct sequence was subcloned into a expression vector pGEX6p-1 and was transformed into E. coli BL21 for expression. The expression fusion protein was purified by GST affinity chromatography, and purified fusion protein was analyzed by Western-blot and ELISA. 72 serum samples were evaluated by ELAISA. A 726 bp gene fragment encoding GRA7 was amplified by PCR, the insertton of GRA7 gene in positive clone was subcloned into pGEX6p-1 to construct a recombinant expression plasmid. The fusion protein with molecular weight 55KD was purified. Recombinant protein GRA7 was recognized by positive toxoplasmosis sera in western blotting and ELISA. Sensitivity and specificity of recombinant protein in comparison with commercial ELISA were 93.75% and 94.64%. The GRA7 gene of T. gondii isolates is highly conservative. The GRA7 is expressed as a recombinant protein in Escherichia coli, which shows an immunoreactivity. The results verify this antigen as a useful tool for diagnostic purposes.

Keywords: Toxoplasma gondii, GRA7, ELISA, recombinant protein





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STUDY ON TOXOPLASMA GONDII INFECTION IN FAULT INDIVIDUALS WITH TRAFFIC ACCI-DENTS

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Toxoplasma gondii is an obligatory intracellular protozoan that results to a broad clinical features disease. Important definitive host of this parasite is cat and the intermediate hosts are warm blooded animals. Mostly, acute toxoplasmosis is considered as a fetus disorder as well as opportunistic disorder in immune-suppressed individuals. In recent years many scientists have considered the serious role of chronic toxoplasmosis in behavior and psychological abnormalities. Some studies have performed on faults in traffic accidents in this issue and various results have been achieved. The aim of the present investigation was to study T. gondii infection in fault individuals with traffic accidents. In our study, sera of 75 inpatient drivers in hospital and 75 controls that were fully satisfied for the study were examined for presence of IgG and IgM antibodies against T. gondii by commercial ELISA kit (Trinity, USA). Results revealed significant difference between IgG levels in two groups (P < 0.05) so that the rate of infection was 62.7% (47 from 75) and 29.3% (22 from 75) in fault individuals and controls without any traffic accident respectively. IgM levels were not significantly different between two examined groups. Although, our results cannot convey the definitive effect of toxoplasmosis on traffic accidents, it sounds the role of tissue cysts in brain and also increasing the level of brain dopamine due to chronic toxoplasmosis - afflicted approximately 30 percent of world population- are noticeable in decreasing the reflexes and decision making of individuals.

Keywords: Toxoplasma gondii, toxoplasmosis, traffic accidents

## PROPRANOLOL ADJUVANTICITY EFFECT COM-PARED TO ALUM ON IMMUNIZATION OF BALB/C MICE WITH PROTEIN COMPONENTS OF TOXOPLASMA GONDII RH STRAIN TACHYZOITES

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Toxoplasma gondii is a cosmopolitan obligately intracellular parasite that infects many species of warm-blooded animals, including humans. The severe or lethal damage caused by T. gondii infection and its high prevalence clearly indicates the need for the development of a vaccine. The ideal vaccine to protect humans against toxoplasmosis would include new adjuvants that elicit a protective TH1 biased-immune response. Proinflammatory cytokines produced by macrophages and TH1 lymphocytes, such as TNF-a, and IFN-y are critical for control of both acute and chronic phases of T. gondii infection. The adjuvanticity effect of propranolol was surveyed and compared with alum as a known permitted human vaccines adjuvant by immunization of ballb/c mice with protein components of T. gondii RH strain's tachyzoites. One hundred Balb/c mice were randomly devided into five groups of 20 mice each, and immunized with phosphate buffered saline (negative control), Toxoplasma lysate antigen (TLA), alum plus TLA (Alum-TLA), Propranolol plus TLA (PRP-TLA) and alum, propranolol and TLA (Alum-PRP-TLA), respectively as groups 1 through 5. Each of these five groups of mice were immunized subcutaneously for three times, at days 0, 10 and 20. Ten days after the last immunization, mice from all groups were divided into three groups. 5 to measure the DTH, 5 to evaluate survival, and 5 to evaluate IFN-γ, TNF-α, lymphocyte proliferation, total IgG, IgG1 and IgG2a. Our results showed that the mice of the PRP-TLA group induced significantly more IFN-y and TNF-a production and lymphocyte proliferation. This group of mice also showed more anti T. gondii IgG2a and DTH responses. After lethal challenge, the mice immunized with PRP TLA, showed a significantly increased survival time. These findings indicate that propranolol as an adjuvant in combination with TLA, can enhance cellular immunity and shift the immune responses to a TH1 pattern.

Keywords: adjuvant, propranolol, *Toxoplasma gondii*, tachyzoite, beta-adrenergic receptor antagonist, vaccine





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#### THE MOLECULAR METHOD IN DIAGNOSIS OF OCULAR TOXOPLASMOSIS IN TEHRAN

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Toxoplasmosis is one of the most prevalent parasitic infections common between human and animals. The disease can be either acquired or congenital. Toxoplasma gondii (T. gondii) is considered as an opportunistic and dangerous infection in immunusuppressed individuals or pregnant women. The parasite reaches to the eve and its retina through circulation and causes irrecoverable chorioretinitis. Attack to the eyes is chronic and sometimes infection relapses and the damaged retina and choroid would not repair. The posterior pole scares can persist a serious threat for the vision. Other symptoms of the disease consist of blurred vision, photophobia, vitreous inflammation, active and passive scares, and clinical signs. Molecular methods are carrying out complementary for recognition of acquired or congenital infections. The aim of the current study is to evaluate efficiency (sensitivity and characteristic) of molecular method in isolation of T. gondii in ocular infections patients with healthy immune systems. Blood samples collected from major ophthalmology centers in Tehran (Farabi, Labbafinejad and Imam Hossein Hospitals). The clinical symptoms were examined and recognized. For examining possible relapse, resampling was done in 15 and 30 days. Thereafter, the buffy coat of the samples was employed for DNA extraction using Diesel Natural Gas (DNG) kit. For performing the polymerase chain reaction (PCR), primer of the gene B1was applied. Following the PCR, from the 71 blood samples, 34 patients (47%) had positive form of the disease. However, with evaluating the first relapse, two weeks after the first step of the disease, among 41 patients, 29 (70%) had positive reaction of the disease. These patients even showed positive PCR in the first step. In the third sampling step (30 days later), 19 patients were examined and 9 (47%) had positive reaction. In other words, in the second and the third steps there were no obvious incidents. The diagnosis of T. gondii, often relys on clinical tests. Nevertheless, unusual clinical symptoms or differentiation of the disease from other alternative symptoms make the diagnosis problematic. However, sensitivity and characteristic of molecular PCR is of great interest. Clinical and complementary methods such as PCR, can be employed for definite and final diagnosis of ocular toxoplasmosis,

Keywords: ocular toxoplasmosis, chorioretinal scar, retinochoroiditis, vitreous inflammation, uveitis, PCR

## A SEROEPIDEMIOLOGIC SURVEY ON TOXO-PLASMOSIS USING ELISA METHOD IN PREG-NANT WOMEN OF BOKAN CITY, NORTH WEST OF IRAN, 2014

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Toxoplasmosis is a common parasitic infection among humans and animals, with a worldwide distribution. The parasite can be transmitted via placenta and causes adverse effects in fetus if the first exposure to Toxoplasma gondii occurs during pregnancy. In this cross-sectional study, 177 pregnant women referred to Bokan medical laboratories were examined serologically for IgG and IgM titers against Toxoplasma infection by ELISA method, after filling a questionnaire. Results were examined by chi-square statistics analysis test. According to the results, 61 out of 177 examined samples (34.46%) were seropositive for anti-Toxoplasma IgG, whereas just one of them (0.56%) was seropositive for anti-Toxoplasma IgM. Several demographic parameters consisting ofage, occupation, habitat, cat and soil contact, nutritional behaviors about the consumption of raw vegetables and undercooked meat (barbecued) were studied in relation to seropositivity using Chi-square statistical test where cat and soil contact, habitat, consumption of raw vegetables in restaurants has significant impact (p<0.05). There was not a statistically significant relationship for other variables (P>0.05).Conclusions: The study showed that in Bokan city, a vast majority of women are seronegative for Toxoplasma gondii infection (65.54%), and thus they may be susceptible to acute Toxoplasma infection during their pregnancy. Therefore, it is recommended to implement a health education program about transmission routes of Toxoplasma for the prevention of congenital toxoplasmosis.

Keywords: toxoplasmosis, ELISA, pregnant, Bokan, seroepidemiology





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## LACK OF INHIBITORY EFFECTS OF FLUPHEN-AZINE AND THIORIDAZINE ON AN AVIRULENT STRAIN OF TOXOPLASMA GONDII IN MICE

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Introduction & Objectives. Toxoplasma gondii is the most common parasite causing latent cerebral infections in human. Due to the long-life persistence of brain T. gondii infections, it is suspected as one of the possible causative agents of chronic neurologic disorders, particularly schizophrenia. Also, it has been shown that some anti-psychotic drugs are able to inhibit the proliferation of this parasite in vitro. There is very limited data regarding the inhibitory effect of anti-psychotics on T. gondii in vivo. In this study, we evaluated anti-Toxoplasma activity of fluphenazine and thioridazine in mice. Mice were divided into six groups: Control, sesame as vehicle, thioridazine (10 and 20 mg/kg), and fluphenazine (0.06 and 0.6 mg/kg). They were inoculated intraperitoneally with brain suspension containing tissue cysts of T. gondii Tehran strain. Three days after inoculation, the mice were injected with sesame and drugs, every other day for three weeks. At the end of second month after inoculation crushed smears were separately provided from whole brain of the mice and the tissue cysts counted with optical microscope. Density of brain cysts is considered as inhibitory index. In data analysis, Kolmogorov-Smirnov test, Analysis of Variance (ANOVA) and Tukey Post-Hoc were used. The tissue cysts were formed in brain of all of the mice, and the mean numbers of brain cysts in the sesame group were higher than control group, but this difference was not significant (p < 0.05). There was not any difference between the numbers of cysts at the two doses of thioridazine compared to control group. Furthermore, there was no difference between the number of cysts of two doses of fluphenazine compared to control and sesame groups.Our study showed that fluphenazine and thioridazine were not able significantly to inhibit T. gondii in vivo.

Keywords: Toxoplasma gondii, fluphenazine, thioridazine, antipsychotic drug, tissue cyst, in vivo

## MOLECULAR DETECTION OF TOXOPLASMA GONDII IN HOUSE SPARROW BY LAMP AND PCR METHODS IN TEHRAN, IRAN

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Toxoplasma gondii is one of the most common zoonotic parasitic diseases in human and warm-blooded animals worldwide. Birds are one of the important intermediate hosts of *T.* gondii. The aim of this study is determination of prevalence of *T. gondii* in the house sparrow by LAMP and PCR methods in Tehran, Iran. A total of 200 sparrows were captured in different regions of Tehran. DNA was extracted from brain and heart samples of each sparrow. LAMP and PCR assays were carried out with a set of primers to detect the 529 bp fragment of *T. gondii*. LAMP and PCR detected *T. gondii* from 17 (8.5%) and 15 (7.5%) of 200 sparrows respectively.These results indicated that sensitivity of LAMP was higher than conventional PCR. Also, these findings provided an insight into epidemiological pattern of *T. gondii* infection in house sparrow in Iran.

Keywords: *Toxoplasma gondii*, sparrow, loop-mediated isothermal amplification (LAMP), PCR, Iran





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PENTYLENETETRAZOL -INDUCED SEIZURE IN MICE INFECTED WITH AN AVIRULENT TOXO-PLASMA GONDII STRAIN IN ACUTE PHASE OF INFECTION

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Epilepsy is a neurological disorder characterized by epileptic seizures. About 20% of cases of epilepsy have no definitive etiology that is considered as cryptogenic epilepsy. T. gondii is suspected as one of the probable etiologies of cryptogenic epilepsy is able to destroy CNS cells in early phase of infection and also following reactivation. Based on epidemiological evidences, there is a significant association between Toxoplasma infections and epilepsy. The present study was performed in order to determine influence of acute (early) infections of T. gondii on pentylenetetrazol (PTZ) -induced seizure in mice. The Balb/c mice were divided into three groups including case (n=24), sham (n=25), and control (n=25). The case group were inoculated intraperitoneally with mice brain suspension containing 20 cysts, sham group inoculated intraperitoneally with mice brain suspension that were negative for T. gondii, and control group did not receive inoculate. Two weeks post-infection, all of the mice were induced seizure with intraperitoneal injections of 75 mg/kg of pentylenetetrazol and latency for tonic, clonic seizures and, generalized tonic- clonic seizure, duration time for tonic and tonic-clonic convulsions, and jumping were recorded. Data were analyzed statistically with ANOVA and Tukey-Kramer tests by SPSS. Latency for tonic, clonic seizures and, generalized tonic- clonic seizure showed a non-significant decrease in case group compared to sham and control groups. Also, duration time for tonic, tonic-clonic convulsion, and jumping did not show significant increase in case group. The present study design cannot demonstrate a significant association between Toxoplasma infections and PTZ-induced seizure in early phase of the infection. A new design for better understanding of the hypothesis is recommended.

Keywords: Toxoplasma gondii, seizure, pentylenetetrazol, mice

#### ISOLATION OF TOXOPLASMA GONDII IN ISFA-HAN REGION USING PARASITOLOGICAL METHODS

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Study of isolated Toxoplasma virulence in mice model should be useful for some prophylactic measures. In the present study the effort was pointed to isolate the organism from suspected human and animal subjects. 20 tissue samples from tonsilectomisied patients, 7 lymph nodes biopsies and 52 placenta or curettage materials were obtained. Blood samples were taken from all of the cases. 11 house mice and 2 cats were captured and tissue and sera samples were taken. All tissue samples were inoculated intraperitoneal into mice according to the Beverley technique. Toxoplasma gondii antibody titers were measured by IFAT and from animals by IHAT. The mice were under observation for 4-6 weeks. Then surviving mice were bled and IHA was used for the detection of Toxoplasma antibody and their brain tissues were examined for presence of Toxoplasma cysts. 23% of tonsilectomised cases, 57.14% cases with enlarged lymph nodes and 50% of blood samples showed a Toxoplasma antibody titer up to 1:200 as measured by IFAT. The antibody titer in cats were estimated to be 1:256 and 1:16384. Toxoplasma gondii was isolated from 2 of 4 mice received lymph node inoculum. The lab mice which received cat's brain and lymph nodes showed antibody titer up to 1:256 by IHAT. The isolated strain was avirulent for laboratory mice and repeated passages had no effects on the virulence of this strain. In previous studies, researchers showed that there were differences between Isfahan region strains with other strains of Iran. More studies is suggested to clarify the biological and virulence characteristics of the parasite in this area.

Keywords: isolation, *Toxoplasma gondii*, Isfahan, parasitological methods





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CORRELATION BETWEEN PERCENTAGE AVID-ITY OF IGG ANTIBODIES AGAINST EXCRET-ED/SECRETED ANTIGENS OF TOXOPLASMA GONDII AND DURING THE COURSE OF TOXO-PLASMOSIS

#### <u>Hossein Abdollahi</u><sup>1</sup>, Behzad Nasiri Ahmadabad<sup>2</sup>, Mohammadtaghi Rezayati<sup>2</sup>, Mohammad Kazemi Arababadi<sup>3</sup>, Ali Fattahi BAfghi<sup>4</sup>

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It has been reported that IgG avidity can be used to distingush chronic toxoplasmosis from acute form. The most suitable Toxoplasma antigens for detection of IgG avidity and its production route are controversial. The main aim of this study was to identify correlation between percentage avidity of IgG antibodies against excretory-secretory antigens (E/SA) of T. gondii and during the course of toxoplasmosis in order to emphasize the suitability E/SA for the differential diagnosis of acute toxoplasmosis. This experimental study was performed using 7 infected in breed Fischer rats. The blood has been collected from infected rats and IgG avidity was measured using E/SA prepared from cell free media. The results demonstrated that avidity index (AI) percentage in sera sample which are collected in days 20 and 30 post infection were significantly increased in comparison to day 7. According to our results, it appears that E/SAs which are prepared from cell free media, as a simple method, can be used for detection of anti-E/SAs IgG avidity to diagnose acute toxoplasmosis.

Keywords: Toxoplasma, IgG avidity, excretory-secretory antigens PREVALENCE OF TOXOPLASMA GONDII IN CHILDREN WITH ATTENTION DEFICIT / HY-PERACTIVITY DISORDERS AND HEALTHY IN PRE-SCHOOL AND SCHOOL LEVEL IN BAM CITY, IRAN

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Toxoplasmosis is one of the most important zoonosis disease caused by parasitic protozoan, Toxoplasma gondii. It is estimated toxoplasmosis infect one third of humans, 90% percent of them have latent form of the disease. The current research shows that Toxoplasma is related with behavioral disorders especially schizophrenia. In this study relation between Toxoplasma gondii and attention deficit / hyperactivity disorders (ADHD) in children of pre- schools and schools level of Bam were studied. In this cross – sectional study, 96 serum samples were collected from ADHD children and 96 serum samples from control group. Sera analyzed by using ELISA test for detecting Ab against Toxoplasma IgG and IgM in two group (case and control). The serological results showed the prevalence of toxoplasmosis in ADHD group was (2.1%) and in control group (2.1%) with identical rate and no significant differences was obtained. The frequency rate of seropositive children of ADHD group contacted with cat were more than others (without contact to cat) and significant differences were seen. The seropositivity of ADHD group for IgG Ab against Toxoplasma in age group under 12 years and among boys and girls were observed with no significant differences. Also, IgMAb against Toxoplasma were not found in ADHD and control group. This study was second research that Anti Toxoplasma IgG were evaluated between ADHD patients and control groups and no significant differences were seen and these results were similar to the first study in Yazd city. More seropositive to toxoplasmosis in ADHD group who had contact with cat indicated important role of cat in toxoplasmosis. In comparison of IgGAb and age group under 12 years, sexes (boys and girls) and level of education of parents for ADHD group, we did not find significant differences. Using more samples and different serodiagnosis tests is recommended for finding the relation of ADHD and toxoplasmosis to propose new strategies in control of ADHD disorders in future.

Keywords:children, toxoplasmosis, deficit / hyperactivity disorders, Bam





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SEROEPIDEMIOLOGICAL STUDY OF ACUTE TOXOPLASMOSIS IN PREGNANT WOMEN IN SHIRAZ IN 2014

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Toxoplasma gondii is an intracellular obligatory parasite that can infect many of warm blood vertebrate animals such as birds, mammals and humans. If transmission occurs in first threesemester of pregnancy, manifestation of the disease is more serious which include abortion, chorioretinitis, hydrocephalia, microcephalia and mental retardation. The current study aimed to investigate the rate of acute toxoplasmosis in pregnant women in Shiraz, during 2014. Two thousands sera samples were collected from pregnant women in Shiraz hospitals affiliated to Shiraz University of Medical Sciences. Demographic data were obtained during sample collection. Toxoplasma IgM ELISA was performed for each sample for detecting IgM antibodies in blood samples. Age of participant was between 20-38 years. Out of 2000 examined samples, five samples were positive for anti-Toxoplasma IgM. Accordingly, the rate of acute toxoplasmosis in pregnant women in Shiraz was 0.25%. Considering the acute toxoplasmosis cases in pregnant women in Shiraz, it is necessary to monitor the mothers during their pregnancy because of the disease importance and the risk of placental transmission to fetus.

Keywords: toxoplasmosis, pregnant women, seroepidemiology, Shiraz

## SEROEPIDEMIOLOGY OF TOXOPLASMOSIS IN PREGNANT WOMEN REFERRED TO IMAM KHOMEINI HOSPITAL IN SHIRVAN CITY, NORTH-WEST OF IRAN

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Toxoplasmosis, is a zoonotic disease in human and animals caused by obligatory intracellular protozoa, Toxoplasma gondii. Toxoplasmosis in humans happens in congenital or acquired forms. Congenital toxoplasmosis caused abortion or mental and physical abnormalities in embryo of women that have no history of contact with parasite before pregnancy. This study evaluated seroepidimiological status of toxoplasmosis among pregnant women referred to Imam Khomeini hospital in Shirvan. In this cross-sectional study, 350 blood samples from pregnant women were collected. Then separated sera were tested for IgG & IgM antibodies with ELISA test. Result: Obtained results were analyzed using SPSS software. 110 cases out of 350 samples were anti-Toxoplasma IgG antibody positive and 12 cases were positive for anti-Toxoplasma IgM antibody. The rate of IgG positive was low and it seems that educational measures before pregnancy about toxoplasmosis is very important.

Keywords: toxoplasmosis, seroepidemology, pregnant womem, Shirvan





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## SEROPREVALENCE OF TOXOPLASMOSIS IN PA-TIENTS REFERRED TO PRIVATE LABORATO-RIES OF CENTRAL PART OF MAZANDARAN PROVINCE

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Toxoplasmosis is a parasitic disease caused by Toxoplasma gondii and is a worldwide health problem more prevalent in socioeconomically deprived populations. Infection of pregnant women through ingestion of sporulated oocysts or tissue cysts of protozoa can result in adverse pathological disorders of fetus and even death. Prophylactic measures, early detection of the infection and treatment can prevent congenital toxoplasmosis and the associated long term complications. The aim of this study was to evaluate the seroprevalence (IgG and IgM titers) of toxoplasmosis in patients referred to medical diagnostic laboratories in three counties of central part of Mazandaran, a northern province in Iran. We also studied socio-spatial risk factors in tested population for toxoplasmosis. This study was carried out using surveying the men and women tested during 12 months (June 2013 to June 2014) in three counties with largest population in central Mazandaran (Amol, Babol and Qaemshahr). The study populations were chosen from people referred to eight medical diagnostic laboratories in these three counties. Demographical and household data such as age, gender, level of education, employment, marital status, number of children and abortion, presence of cats in and around the house as well as their address were obtained via questionnaire forms submitted at the time of testing. IgG and IgM developed against Toxoplasma antigens were determined using enzyme immunoassay. IgM or IgG positive results were considered as positive for Toxoplasma infection. Classification analysis was performed using machine-learning algorithm ('random Forest' package). The comparison between groups were done using Pearson's Chi-squared test. All statistical analysis was performed in R (Version 3.1.2). The results showed that 257 out of 393 patients referred to laboratories (65.4%) were seropositive either by IgG or IgM assays. The rate of infection in rural areas was (1.2 folds) more than urban areas. Among the three counties, Amol showed significantly the highest seroconversion followed by Qaemshahr and Babol. In addition to location and county the patients belonged to, we found that presence of cats around the house can significantly affect the seropositivity. On the other hand, the results did not show significant effect of other variables on seropositivity.Since farming is the main occupation in rural areas of Mazandaran province, close contact with soil (as the source of oocyst of T. gondii), may explain the increased prevalence in rural versus urban areas. Furthermore, lack of proper hygiene in rural areas may contribute to the infection status. We have also found that the people with a "prior to diploma" education, have the highest rate of seropositivity followed by Diploma, BSc, illiterate and MSc. We expect that these preliminary data, will contribute to development of an integrated program for prevention of congenital toxoplasmosis adapted to the population of Mazandaran province. Nevertheless, there is a need for an educational program and further epidemiological studies to understand the health impact of toxoplasmosis in women of child-bearing age, congenital malformation in her infant, and particularly in undereducated women.

STUDY ON CHANGES OF TROPONIN AND CRE-ATINE PHOSPHOKINASE IN EXPERIMENTAL INFECTION WITH TOXOPLASMA GONDII IN MICE

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Toxoplasma gondii is an obligate intracellular parasite with widespread distribution. The parasite is considered as one of the factors of myocarditis and heart destructive tissue, so the aim of this study was to investigate possible role of T. gondii in presence of certain biomechanical enzymes in rats sera which infected with the parasite and the rats that afflicted with chronic T. gondii then became immunosuppressed. In this study, 36 rats were experimentally infected with T. gondii and the second group of 12 rats served as non-infected controls. Blood sample were taken in different days from 15 rats and 7 controls before injection of dexamethazone and also from 21 rats and 7 controls after injection of dexamethazone and then biochemical factors of them were compared. The results showed that serum levels of CPK at day 7 post infection and before injection of dexamethasone was higher than other days and average serum level of controls (p < 0.05). Also the results revealed that serum levels of CPK in days 11 and 12 post injection of dexamethazone in infected rats increased more significantly than other days and average of controls (p<0.05). Results showed that serum levels of troponin I have got significant increase in day 10 day post infection and also days 11 and 12 after dexamethazone injection than other days and average of controls (p<0.05). The results of the study showed that Toxoplasma in acute phase may cause changes in enzymes associated with heart failure. These findings can interfere with detection of other cardiac lesions that enhance these enzymes.

Keywords: troponin I, CPK, Toxoplasma gondii, rat

Keywords: toxoplasmosis, seroprevalence, Mazandaran, Iran





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 A SEROEPIDEMIOLOGICAL STUDY OF TOXO DETEC

 PLASMOSIS AMONG FEMALE STUDENTS OF
 STRAY CA

 SABZEVAR UNIVERSITY OF MEDICAL SCIENCES
 IN 2013-2014

#### <u>Hossein Elyasi</u><sup>1</sup>, Mohamad Shafie Mojadadi<sup>1</sup>, Najmeh Mahmoudabadi<sup>2</sup>, Rahim Golmohamadi<sup>3</sup>, Melika Laal Kargar<sup>2</sup>, Zohreh Sajadinia<sup>2</sup>

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Toxoplasma gondii is an obligate intracellular protozoan parasite that infects all warm-blooded animals including humans. Acute Toxoplasma gondii infection in pregnant women frequently leads to congenital toxoplasmosis which may cause abortion or severe diseases such as premature birth, permanent neurological damage and visual impairment. Serological survey in females before marriage can reveal immune status of them against toxoplasmosis. The aim of this research was to determine the anti Toxoplasma antibodies in female students of Sabzevar university of Medical Sciences. In this cross-sectional study, 137 blood samples were taken from female students that were randomly selected and used for measurement of anti Toxoplasma antibodies by use of ELISA Method. IgG antibodies were detected in 18 (13.1%) samples, No IgM antibody was found.Rssults of present study revealed that 86.9% of female students were seronegative and at high risk for acquired infection, thus they should be educated to prevent the congenital toxoplasmosis during the period of pregnancy.

Keywords: seroepidemiology, toxoplasmosis, female, student, Sbzevar

DETECTION OF TOXOPLASMA GONDII IN STRAY CATS (FELIS CATUS) USING GRA6 GENE, TEHRAN

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Toxoplasmosis is an endemic disease worldwide and a major public health concern for humans and domestic animals. It is caused by the parasitic protozoan Toxoplasma gondii. Cats and wild felidae play a crucial role in the epidemiology of this disease; they are the only definitive hosts and are therefore the only ones to shed oocysts in their feces. It is generally assumed that cats play a major role in transmitting T. gondii through the fecal contamination of soil, food or water since they may excrete millions of oocysts over a period of 1-2 weeks. Rather than the consumption of raw or undercooked meat, the ingestion of vegetables or water contaminated with oocysts is currently thought to be the most common route through which humans become infected. It is therefore important to detect cats that are shedding oocysts if control of the disease is to be achieved. In this study the prevalence of Toxoplasma gondii was surveyed by using a nested polymerase chain reaction (nPCR) that was targeted to T. gondii GRA6 gene in stray cats. Biological samples were collected from 41 stray cat that died in accidents or other reasons from different areas of Tehran. The brain of each cat was then removed for direct parasite detection in sterile condition and DNA was extracted with phenol-chloroform method. The 344 bp of GRA6 gene amplified by Nested-PCR method to confirm the presence of Toxoplasma gondii DNA. Results showed, 15/41 cats (36. 6%) were infected with Toxoplasma gondii. This study used brains from stray cats for direct detection since this organ is that most frequently affected. PCR is a sensitive, specific and rapid alternative for detecting T. gondii; Indeed, PCR can detect the DNA of parasites even when the tissues available for testing are in state of decomposition. Because of high occurrence of T. gondii infection in cats in this study, cats may play a serious role in human and other mammalian toxoplasmosis in Tehran.

Keywords: Toxoplasma gondii, Nested-PCR, GRA6, stray cats





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#### SEROPREVALENCE OF TOXOPLASMA GONDII INFECTION IN HOUSEHOLD DOGS, IN URMIA, NORTHWEST IRAN

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Toxoplasma gondii is an obligatory intracellular protozoan which has a broad range of intermediate hosts, consists of about all warm-blooded animals (mammals and birds). Toxoplasmosis has cosmopolitan distribution and has been reported in humans and different animals from most areas of Iran. The present study was conducted to evaluate the rate of anti-Toxoplasma IgG in household dogs, in Urmia. Ninety three blood samples were collected from clinics which had obtained from dogs for different hematology/biochemistry tests in a 4 months period in 2011. The samples were examined for presence of anti-Toxoplasma IgG, using ELISA method. The results showed that 57 out of 93 samples (61.3%) were positive for Anti-Toxoplasma IgG. Twenty seven (47.4%), 23 (40.3%) and 7 (12.3%) of the infected dogs belonged to the age groups of; less than 1 year, 1-4 years and more than 4 years, respectively. Also 25 (43.9%) cases of the infected dogs were male whereas 32 (56.1%) were female. In spite of quantitative variations in the rates, statistical analysis showed no significant difference about the age groups, sex and breeds (P>0.05). Furthermore, there was no significant difference about the dogs' life style rates (Indoor versus Outdoor (50%-62.65%) kept dogs) (P>0.05). The rate of infection in the dogs with free roaming (65.43%) was significantly more than the other dogs (P<0.05). Similarly, there was a significant difference (P<0.05) in the infection rates of the dogs with the cooked food diet (50%), row food diet (90%) and the mix food diet (70.96%). According to the results, it can be concluded that, as the dogs' infection to Toxoplasma has the high rate in Urmia, it is necessary to consider it as a probable effective agent in differential diagnosis of the cases. Also it can be concluded that, the free roaming behavior and the type of the diet are the only parameters which can affect the rate of infection in studied dogs.

Keywords: toxoplasmosis, ELISA, dog, Urmia, Iran.

#### MOLECULAR DETECTION OF TOXOPLASMA GONDII IN CROWS (CORVUS) OF MIYANEH CITY

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Toxoplasma gondii infections are prevalent in many avian species and can cause mortality in some bird hosts. Although T. gondii has been isolated from various species of birds, the role of many different species of wild birds such as scavenger birds that feed on animal and their possible role in the epidemiology of toxoplasmosis remain unknown. Among these, crows is a bird omnivores and a large part of their diet provides Carrions, that well adapted to living alongside humans and in some cities there is a large population. The present study reports the presence of T. gondii DNA by PCR in brain tissues of free ranging crows (Corvus cornis, Corvus frugilegus, Pica pica, Pica hudsonia) from Miyaneh, a city in East Azerbaijan province, Northwest Iran. In this study, 50 crows were captured from different areas of Miyaneh city. DNA was extracted by a conventional phenol-chloroform procedure from brain of crows and was evaluated by Nested Polymerase Chain Reaction (nPCR) assay for detection of the T. gondii dense granule antigen GRA6 gene. The finding indicated that 17 out of 50 (34%) samples were positive by nPCR. The high prevalence in these birds species suggests that infected crows may be responsible for widespread infection of scavenger birds which may further transmit infection to other carnivorous intermediate hosts or feline definitive hosts when consumed post-mortally. In addition, the host can be considered as a resource to maintain the cycle of parasite transmission. Infected carrion is likely to be responsible for widespread infection of carcass scavenger birds which may further transmit infection to other carnivorous intermediate hosts or feline definitive hosts when consumed post-mortem

Keywords: Toxoplasma gondii, nested-PCR, GRA6, crows





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MOLECULAR AND PATHOLOGICAL SURVEY ON TOXOPLASMOSIS IN ABORTED FETUS OF SHEEP AND GOAT IN KURDISTAN PROVINCE, IRAN

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Introduction & Objectives; Toxoplasma gondii is an obligate intracellular protozoan, in the Apicoplexa phylum, causes Toxoplasmosis, which is the main cause of abortion and congenital infection in sheep and goats. Various techniques are used to detect the pathogen in infected sheep fetus, one of the most sensitive and more specific diagnostic technique is modified Nested-PCR using gene B1 organism. The purpose of this study was to determine toxoplasmic abortion in sheep and goat by using PCR and histopathological tests in years 2012-2013 in the province of Kurdistan. To identify examples of positive and negative after PCR amplification of the first and second PCR products on an agarose gel (2%), electrophoresis (the first step to identify the band 193bp and secondly to identify band 96bp) revealed 10 positive samples, the first step 3 samples were 193b band and the second band was 96bp.96bp band of the samples were positive only in the second phase and negative examples. 8.3 % of sheep and 10% of fetal goat embryos were infected with T. gondii. All positive samples were isolated from embryos that have more than 2.5 months of age. In positive PCR sample, histopathological evaluation, for presence of cysts or non suppurative encephalitis were implemented. In two cases, symptoms such as edema, hyperemia, gliosis, non purulent meningitis was found ,andin negative PCR result, cases in histopathological studies, too, no cysts was found. According to these results Toxoplasma gondii is known as a cause of abortion in this province due to prevalence of infection in livestock such as sheep and goast, and also as a source of infection in humans.

Keywords: Toxoplasma, sheep, goat, molecular biology, Kurdistan

## SEROEPIDEMIOLOGY AND RISK FACTORS OF TOXOPLASMOSIS AMONG WOMEN REFERING TO HOSPITAL OF KURDISTAN

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Toxoplasma gondii is the obligatory intracellular protozoan parasite in Apicomplexa phylum and one of the important zoonotic parasites with worldwide distribution. Human can be infected by eating raw meat infected with cyst and water infected by oocyst or by vertical transmission. This cross sectional study was conducted on 164 pregnant women referred to medical centers. Demographic data and risk factors were collected using a questionnaire. Also, blood samples were taken from each subject to detect IgG antibodies by ELISA method. Data was entered in to SPSS 16 software and descriptive statistics, chi-square were used for analysis. The Lowest and highest Ab titer were reported in 35-39 age and 15-19 age groups, respectively. No significant relation was seen between seropositivity and age. No significant relation was seen between seropositivity in urban and rural women. All women have consumed vegetables.

Keywords: *Toxoplasma*, Kurdistan, pathology, molecular biology, serology





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## PREVALENCE OF TRICHOMONAS VAGINALIS INFECTION IN KASHAN, IRAN

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Trichomonas vaginalis infection is one of the most common sexually transmitted diseases in humans. T. vaginalis is a parasitic protozoa with a predilection for human urogenital tract and causative agent for vaginitis, cervicitis and urethritis in females. T. vaginalis infection is associated with risk of Human Immunodeficiency Virus infectivity and pregnancy complication. In this study, the prevalence of T. vaginalis in individuals referred to public health units in Kashan, Iran was investigated. This study was conducted on 970 women and 235 men referred to 5 health centers in Kashan, during October 2012 to August 2013. Demographic data were collected as the study protocol. Vaginal discharges and urine samples were obtained and examined by Trypticase-Yeast Extract Maltose (TYM) culture medium and wet-mount methods. The prevalence of T. vaginalis was determined using culture based method and wet-mount examinations. The overall prevalence of trichomonal infection was 2% (95% CI,  $2\pm0.08$ ). The age of infected individual was 33.7±9.4 years. All of those infected were married housewives and 58.3% of them had primary school education. No statistical correlation was observed between clinical manifestations and parasitological results (P= 0.8). This study showed a relatively low prevalence of T. vaginalis infection in the study population. Since the clinical signs of trichomoniasis are the same as other Sexually Transmitted Diseases (STDs), confirmatory laboratory tests are necessary. Due to adverse outcomes of the disease, public education regarding implementation of personal hygienic measures and avoiding of inappropriate sexual contacts is highly recomended.

Keywords: *Trichomonas vaginalis*, vaginal discharge, culture, prevalence, Iran

## EFFECT OF AQUEOUS AND ALCOHOLIC EX-TRACTS OF PELARQONIUM ROSEUM ON THE GROWTH OF TRICHOMONAS VAGINALIS IN VITRO

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Trichomoniasis is the most common sexually transmitted parasite in the world. The mainstay treatment for trichomoniasis is metronidazole, but some drug resistant strains have also been detected. Considering the therapeutic properties of Pelargonium roseum in traditional medicine, this study aimed to examine the anti-trichomonal effects of Pelarqonium in vitro. In this experimental study, 1203 subjects referred to Kashan medical centers were randomly tested for Trichomonas vaginalis, of them 23 were infected with T. vaginalis. Three T. vaginalis isolates were cultured in a TYI-S-33 medium and the effect of Pelargonium extract was investigated on them. Different concentrations (12.5, 25, 50, 100, 200, 400, 800, 1000  $\mu g/ml$ ) of *Pelargonium* extracts were tested in three different times (24, 48 and 72h) on T. vaginalis trophozoeites. Also the effect of metronidazole (0.025, 0.05, 0.1, 0.2, 0.4  $\mu$ g/ml) was investigated as the drug control. In all tests the numbers of live and dead parasites were counted by trypan blue staining. The findings showed that the alcoholic and aqueous extracts of *Pelargonium* have inhibitory effects on the growth of T. vaginalis trophozoeites. The IC50 of the aqueous and alcoholic extracts of *Pelargonium* and metronidazole after 24h were 54.67, 27.63 and 0.0326, respectively. While the alcoholic and aqueous extract of Pelargonium have anti-Trichomonas effect, the anti-Trichomonas properties of alcoholic extract is more than its aqueous extract. Since Pelargonium is a herbal drug, it can be tested in vivo.

Keywords: Trichomonas vaginalis, alcoholic extract, aqueous extract, Pelarqonium roseum, in vitro





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EFFECT OF 3'-DEOXYADENOSINE & DEOX-YCOFORMYCIN ON HEMATOLOGICAL PARAM-ETERS AND ACTIVITY OF ADENOSINE DEAMI-NASE IN INFECTED MICE WITH TRYPANOSO-MA EVANSI

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The Trypanosoma evansi is the etiologic agent of trypanosomosis, a disease with broad distribution in Africa, Asia, and Latin America which may result in immeasurable economic losses. It is important to investigate alternatives to improve the success of the treatment using new drugs, anti-protozoa associations, and other. Based on this idea, some studies have emerged suggesting new options for the treatment of trypanosomosis. Seventy animals were divided into seven groups, which were divided into two subgroups each for sampling on days 4 and 8 post-infection (PI). The groups were composed of three uninfected groups (A-C), namely, not-treated (A), treated with 3'-deoxyadenosine (B), and treated with deoxycoformycin (C) and four infected groups, mice with T. evansi (D-G), namely, not-treated (D), treated with 3'deoxyadenosine (E), treated with deoxycoformycin (F), and treated with a combination 3'-deoxyadenosine and deoxycoformycin (G). Hematological parameters and ADA activity were evaluated in plasma and brain. Animals in groups B and C exhibited a reduction in the levels of plasma total protein compared group A. Animals in groups D and F showed changes in the hematological parameters. The ADA activity significantly reduced in the animals of groups C, D, F and G. Mice in the group E presented increased ADA activity in plasma. Therefore, we conclude that the treatment interferes significantly in the hematologic parameters in mice infected with T. evansi. On the other hand, when the ADA inhibitor was used, we observed a significant decrease in the values of hematocrit, total erythrocytes, and hemoglobin concentration. The deoxycoformycin was able to inhibit the ADA activity of parasite thus it may be one of the mechanisms of efficacy of this treatment.

Keywords: *Trypanosoma evansi*, 3'-deoxyadenosine, deoxycoformycin, hematology, adenosine deaminase

## TRICHOMONAS VAGINALIS IN A WOMAN FROM MAZANDARAN PROVINCE

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Trichomoniasis, caused by protozoan *Trichomonas vaginalis*, is urinary tract infection of male and female genitalia. It is one the most important and neglected sexual transmitted infection (STI) in the world. We report a 45-year-old woman, living in Ghaemshahr district from Mazandaran province, referred to Razi hospital with chief complaint including foamy discharge, vaginal itching, painful urination, especially when dealing with an inflated position. *Trichomonas vaginalis* found on routine urinalysis. The patient was treated with metronidazole and had complete resolution of the infection. This report offers awareness rising of gynecologists regarding *T. vaginalis* infection as a neglected STI among women in this area of Iran.

Keywords: trichomoniasis, *Trichomonas vaginalis*, sexual transmitted infection, Mazandaran





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A DIAGNOSTIC AND SYMPTOMATOLOGICAL STUDY ON TRICHOMONIASIS AND OTHER FORMS OF VAGINIOSIS IN SYMPTOMATIC PREGNANT WOMEN IN RAFSANJAN, SOUTH CENTRAL IRAN, IN 2012-2013

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Trichomonas vaginalis, the causative agent of trichomoniasis, is responsible for more than half of all sexually transmitted infections (STIs). The present study aimed to elucidate the ethiologic agents of vaginosis, the status and clinical manifestations of T. vaginalis infection in symptomatic pregnant women in the area and to evaluate the different diagnostic methods. A total of 162 pregnant women with at least one sign or symptom of vaginosis, referred to two gynecologic and obstetrics clinics in Rafsanjan, were randomly selected. Through speculum examination of patients by gynecologists, clinical diagnosis was determined, vaginal discharge was collected by using two sterile cotton swabs from the posterior fornix and vagina pH was measured. Samples were examined by three diagnostic methods including wet mount, culture in TYI-S-33 medium and polymease chian reaction (PCR). T. vaginalis was detected in 19.4%, 27.2%, 56.2% and 51.6% of subjects according to diagnostic methods of clinical diagnosis, wet mount, culture and PCR, respectively. There was statistically significant relationship between T. vaginalis infection and patients' age, gestational age, marriage age, residence, educational level, parity. The symptomatological pattern in the 91 women infected with T. vaginalis was as follows: leukorrhea, 96.7%; urine frequency, 65.9%; odorous secretion, 63.3%; urogenital itching and irritation, 53.8%; vaginal inflamation, 47.3%; Dyspareunia, 39.6%; and dysuria, 16.5%. Our results indicated a high prevalence of T. vaginalis in symptomatic pregnant women and very low sensitivity and relatively high specificity of clinical diagnosis and wet mount technique compared to culture and PCR. We also conclude that, pregnancy increases the susceptibility to the infection in a gestational age-dependent manner.

Keywords: symiology, Trichomonas vaginalis, diagnosis

## COMPARATIVE STUDY ON THE PREVALENCE OF TRICHOMONIASIS IN THE PIGEON AND TURKEY IN ZABOL DISTRICT, IRAN

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Although the infection with Trichomonas gallinae is very prevalent in the pigeon worldwide, it rarely occurs in turkey. The aim of the present study was to determine the prevalence of this parasitic disease among the native pigeons and turkeys in Zabol district of Iran. During this study (from February 2013 to August 2014) 300 wet swab samples were collected from crop of pigeon (no= 150) and turkey (no= 150). All of the samples were investigated directly under light microscope to find motile Trichomonas gallinae. Of the 150 pigeons, 72.7% (n =109) and among 150 turkeys, 0.7% (n=1) were infected with the parasite. Our finding showed a significant difference in the incidence of the disease in turkeys and pigeons (P < 0.001). The present result is the first observation of trichomoniasis in turkey in Iran. Like many parts of the world the infection is very prevalent in pigeon population in Zabol district of Iran.

Keywords: Trichomonas gallinae, pigeon, turkey, Zabol, Iran





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IN VITRO ASSESSMENT OF TRICHOMONAS VAGINALIS TOLERANCE TO BROAD-SPECTRUM ANTIBIOTICS

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The advances acquired in the cultivation of protozoa have provided the possibility to investigate on the biology of parasites including development, immunology, biochemistry and susceptibility. There are some issues about axenisation of protozoan parasites and so far we have overame on some of them but not all. One of the problems is eradication of refractory microorganism, associated with protozoa, in axenic culture. The aim of this study was to determine the endurance and tolerance of Trichomonas vaginalis protozoan to some relatively broad-spectrum antibiotics. Aerobic and anaerobic drug susceptibility testing was carried out on five T. vaginalis strains preserved in the research laboratory of parasitology of Hamadan University of Medical Science. Drugs powder of gentamicin, chloramphenicol, ciprofloxacin and amphotericin B was dissolved in distilled water sterilized through filtration (0.22 µm pore size) and serial twofold dilutions, ranging from 1 to 8200 µg/ml, were prepared by using medium culture. Trichomonad cells, in the logarithmic phase of growth,  $1 \times$ 105 and 5  $\times$  103 trophozoites per well were utilized for aerobic and anaerobic assay, respectively. The minimum inhibitory concentration (MIC) was determined after 24 and 48 hrs under aerobic and anaerobic incubation at 35.5°C. The lowest concentration of the drugs in microplate wells in which no motile parasites were detected, considered as MIC. For MIC anaerobic tests, the test microplates were placed in anaerobic jar containing Anaerocult C pack (Merck) for generation of an anaerobic environment. All susceptibility tests of each isolate were performed two times in triplicate in both conditions compared with control. The mean MICs  $\pm$  standard deviation of the drugs were determined after 24 and 48 hrs aerobic incubation, respectively, as follows: gentamicin:  $5880 \pm 2300$ ,  $2310 \pm 1150$ ; chloramphenicol:  $1890 \pm 469$ ,  $1680 \pm 575$ ; ciprofloxacin:  $942 \pm 240$ ,  $942 \pm 240$ ; amphotericin B: 11.2  $\pm$  4, 11.2  $\pm$  4. In anaerobic condition, the mean MICs  $\pm$  standard deviation of the drugs were as followed: gentamicin:  $1470 \pm 575$ ,  $1260 \pm 469$ ; chloramphenicol:  $409 \pm$ 140, 307  $\pm$  114; ciprofloxacin: 153  $\pm$  57, 153  $\pm$  57 and amphotericin B:  $11.2 \pm 4$ ,  $11.2 \pm 4$  after 24 and 48 hrs incubation, respectively. The results showed that amphotericin B is more toxic than the other drugs used in this survey and the trichomonad parasite can tolerate high concentration of gentamicin. Thus, according to the results, the antimicrobial agents can be considered as a potent antibiotic for achieving the axenic cutler of Trichomonas vaginalis parasite.

Keywords: Amphotericin B, Chloramphenicol, Ciprofloxacin, Gentamicin, *Trichomonas vaginalis*  FREQUENCY OF TRICHOMONIASIS AMONG WOMEN IN TEHRAN, HAMADAN AND TOYSER-KAN, NORTH AND WEST OF IRAN, 2011

### <u>Mohammad Matini</u><sup>1</sup>, Mostafa Rezaeian<sup>2</sup>, Mahdi Mohebali<sup>2</sup>, Amir Hossein Maghsood<sup>3</sup>, Mohammad fallah<sup>4</sup>, Soghra Rabiee<sup>3</sup>

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Infection with Trichomonas vaginalis, an anaerobic protozoan parasite, is one of the most nonviral sexually transmitted diseases (STDs) worldwide that involve millions of individuals annually. Trichomoniasis considered as a sign for other STDs can be accompanied with serious sequelae such as adverse outcome pregnancy, predisposition to cervical cancer and increased susceptibility to HIV infection. This study was conducted to determine the frequency of Trichomonas vaginalis infection in women in Tehran, Hamadan and Toyserkan, north and west of Iran. This study was performed on 950 suspected women referred to gynecology clinics in Tehran, Hamadan and Toyserkan, during 2011. To detect T. vaginalis parasite, two vaginal swab samples were taken from participants. The first swab was examined by light microscopy at low (×100) and high (×400) powers and the second subjected to Dorset's culture. The culture medium was tested daily up to 7 days until they turned positive. Outcomes of two diagnostic methods demonstrated 45 infected individuals for T. vaginalis (6.2%), although thirty seven of patients (5.4%) were detected only by the wet mount technique. In terms of geographical distribution, trichomoniasis was at high frequency in Toyserkan (8.9%) despite Hamadan (3.1%) and Tehran (3.4%). All women suffered from at least one of the signs and symptoms of trichomoniasis. Vaginal discharge was the most prevalent signs of trichomoniasis (100%) observed in the participants. Other signs and symptoms included itching, dysuria, dyspareunia and inflammation of the genital tract. Conclusions: Frequency of trichomoniasis is considerable in the society and because of medically important complications of infection, there is a great need for effective comprehensive sexuality education programs in the community.

**Keywords:** trichomonasis, frequency, Malayer, Tehran, Hamadan





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FREQUENCY OF TRICHOMONAS VAGINALIS INFECTION IN PREGNANT WOMEN REFERRED TO HEALTH AND MEDICAL CENTERS IN AR-DABIL CITY, 2013-2014

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Trichomoniasis, a protozoan infection with the prevalence of more than 170 million people worldwide, is a widespread infection which is transferred through sexual contact. Trichomonas vaginalis in women causes pelvic inflammatory disease, increased risk of fallopian tube dependent infertility, ectopic pregnancy, preterm labor, the birth of low weight infants and the increase in the possibility of HIV transmission. Considering the importance of Trichomonas vaginalis infection in pregnant women and lack of data from Ardabil city, this study was performed to identify this infection in pregnant women referred to health and medical centers. Vaginal discharge from 500 pregnant women was collected with sterile swap and disposable speculum and examined for Trichomonas vaginalis by direct microscopic examination and cultured in Diamond specific medium. A testimonial and questionnaire were completed for each case and the results were analyzed using descriptive and Chi-Square tests and by SPSS statistical software version 19. In this study, the culture of samples displayed 12 positive cases (2.4%). Furthermore Trichomonas vaginalis trophozoites were observed in five cases (1%) with microscopic examination. Among different clinical manifestations there was a significant relation between discharge and the infection. The present survey confirmed the frequency of Trichomonas vaginalis infection in pregnant women in this rigion. Therefore our results highlight the importance of preventive intervention in this group.

Keywords: Trichomonas vaginalis, trichomoniasis, pregnant women, Ardabil

## STUDY OF THE PREVALENCE OF TRICHOMO-NAS VAGINALIS IN WOMEN REFERRED TO HEALTH CENTERS IN DEHLORAN COUNTY

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Trichomonisis is one of the most prevalent urogenital sexually transmitted diseases. Trichomonas vaginalis is flagellated protozoa living in human urogenital tract. This parasite has a trophozoite form and propagates via longitudinal binary fission. Asymptomatic infected people work as carrier causing distribution of the disease and therefore have epidemiological importance. Since determination of trichomoniasis prevalence rate can be warning of STD distribution and because no similar study was carried out in the Dehloran County we decided to evaluate the prevalence rate of the parasite in the region. In this descriptive study 600 women referred to health- treatment centers were visited for vaginal and cervix by speculum. Samples were collected by cotton swaps and dissolved in 1 ml PBS. Wet smears were prepared from the solutions, stained with Gimsa and studied directly under light microscope. The Trichomoniasis prevalence rates were 14% in condom users, 12% in IUD users, 40% in users of natural way to prevent pregnancy and 34% in women who used other contraceptive methods. The highest rate of trichomoniasis was detected in women who used natural way to prevent pregnancy. The lowest rates of trichomonisis was detected in women who used IUD and condom for contraceptive purpose. It seems that using condom is the safest method for prevention of the trichomoniasis.

Keywords: trichomoniasis, prevalence, Dehloran, women





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#### PREVALENCE OF TRICHOMONAS VAGINALIS INFECTION IN WOMEN ATTENDED AT GYNE-COLOGY CLINICS IN TEHRAN, IRAN

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Trichomoniasis is one of the most common sexually transmitted disease in the world. Trichomonas vaginalis is the cusative agent of this infection and the third common cause of vaginitis in women. Millions of males and females have to visit the STD clinics due to infection with this parasite per year. An important issue about this protozoa is diagnosis and treatment of the patients and their sexual partners. Different methods are available to detect this parasite but due to high sensitivity of PCR method, the present study aimed to investigate the prevalence of trichomoniasis in women referred to gynecology clinics in Tehran.In this cross-sectional study, two vaginal swabs and urine sample was collected from each patient to the clinic with their due consents. One swab was used for cultivation in TYI-S-33 media and the second sample kept in saline and transferred to the laboratory. The DNA of the parasite extracted by DNG+ kit and then PCR was conducted by specific primers of P270 gene that were present in all isolates. From 100 patients participated in this study, 37 cases were reported as suspected to infection with T. vaginalis during per speculum examination by gynecologist. By PCR method, 8 (8%) samples were diagnosed and 6 (6%) samples by culture media were infected with T. vaginalis in presence of positive control. Other suspected cases (29) suffered from vaginal infections by other microorganisms. The results of this study indicated that, there is significant difference between the clinical diagnosis and molecular techniques. Therefore, clinical symptoms alone cannot be used for diagnosis of diseases, such as vaginitis, for trichomoniasis. Complementary methods such as direct microscopy examination with low sensitivity can help the physicians in proper diagnosis. Inappropriate use of drugs due to incorrect diagnosis brings about drug resistance and economic loss that is one of the most important problems in our country.

MOLECULAR TYPING OF THE PFOB GENE OF TRICHOMONAS VAGINALIS ISOLATES BY PCR– RFLP IN KARAJ CITY, ABORZ PROVINCE, IRAN

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Trichomonas vaginalis is a human urogenital pathogen that causes trichomoniasis, the most common nonviral parasitic sexually transmitted infection in the world. Because of disease importance in public health and controversial ideas about the prevalence of drug resistance and that the PFOB gene is involved in resistance, this study was carried out. Forty five clinical T. vaginalis isolates of vaginal secretions and urine sediments were collected from Alborz Province since 2012 till 2014. Samples were cultured and DNA was extracted and PFOB gene was amplified by PCR technique. All samples were positive. Eight samples were sequenced to determine the size of the fragments and to confirm the banding patterns. The sequences edited and aligned and compared with reference sequences from GenBank. Then all PCR products were analyzed by RFLP methods with Mnl1 and Taq1 restriction enzymes. Digestion of the amplified product with Mnl1 and Taq1 restriction enzymes yielded two and three patterns, respectively. The results revealed four types. Of 45 isolates, 10 samples (22.2%) were Type 1, 14 samples (31.1%) Type 2, 11 samples (24.4%) Type 3 and 10 samples (22.2%) Type 4.Alignment of the sequences showed that sequence types differed from the reference strain by five nucleotide position. Four of them, nucleotides 24, 249, 567 and 738 were silent mutation. Just mutation happened at position 955 of the PFOB gene in which the alanine was replaced by proline in Accession Number KF747379

Keywords: *Trichomonas vaginalis*, PCR-RFLP, PFOB gene, Karaj, Iran

Keywords: trichomoniasis, STD, prevalence, P270 gene





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GENOTYPIC DESCRIPTION OF TRICHOMONAS VAGINALIS ISOLATED FROM WOMEN REFERED TO TABRIZ HEALTH CENTERS USING ACTIN GENE BY PCR-RFLP METHOD IN 2013-2014

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*Trichomonas vaginalis* is a felagelated and anaerobic protozoa that causes urogenital infection in human. Trichomoniasis is the most prevalent nonviral sexually transmitted disease all over the world. In this study, genotypes of the parasite were determined in the infected patients. Totally, 50 samples of *Trichomonas vaginalis* were isolated from the patients and cultured in diamond's medium. Total genomic DNA was extracted from all isolates with Takapuzist Extraction kit using specific primers, PCR amplification was performed and the products were digested with restriction endonucleases Hind2, Mse1 and Rsa1. In this study, 64% of samples were genotype G and 36% were genotype E.According to the results, analyzing of Actin gene using PCR-RFLP method is recommended for genetic description of *Trichomonas vaginalis*.

Keywords: Trichomonas vaginalis, actin gene, PCR-RFLP

STANDARDIZATION OF ISOENZYME CHARAC-TERIZATION OF TRICHOMONAS VAGINALIS ISOLATED FROM PATIENTS IN SHIRAZ AND KERMAN, IRAN

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Trichomoniasis is a sexually transmitted disease which is distributed in various parts of the world and is a urogenital system parasite. The causative agent is Trichomonas vaginalis, a flagellated protozoa. Trichomoniasis has different symptoms including yellow - green discharge, puritis, vaginal discharges malodor and abdominal pain. A variety of immunological, serological and pathological tests have been used to characterize different strains of Trichomonas vaginalis. During the last few years, some differences have been defined and very few data are available on the intraspecific variation in the biochemical activities of trichomonads and the relationship of these varieties to other biological characteristics. Isoenzyme technique for the identification of Trichomonas vaginalis is essential for clarifying the epidemiological, pathological and biological aspects of trichomoniasis. Specimens of vaginal fluid and urine samples were collected from 480 female and male patients referring to health centers and hospitals located in Shiraz and Kerman cities in the south of Iran. All samples were examined by light microscope for diagnosis based on morphological characteristics and jerky movements in wet mount preparations. Five enzymatic systems including malate dehydrogenase (MDH), malic enzyme (ME), glucose 6 phosphate dehydrogenase (G6PD), phosphoglucomutase (PGM) and glucose phosphate isomerase (GPI) were used for identification of isolated organisms. The findings showed that the TYI - S - 33 culture medium was the best medium in comparison to CPLM and Diamond media for isolation and mass cultivation of Trichomonas vaginalis so that 35, 18 and 8 cases were isolated in these media, respectively. Isoenzyme electrophoretic patterns showed two different zymodems in studied isolates. The isoenzymic patterns of Shiraz and Kerman cases were compared.In this study 2 zymodems were observed. There are some evidences about heterogeneity of these urogenital protozoa in the world. It can be suggested that each zymodems may be responsible for pathogenesis and immunological characters of parasite. We suggest more study in this field for clarifying more details about this sexually transmitted parasite.

**Keywords:** *Trichomonas vaginalis*, culture media, TYI –S-33, isoenzyme electrophoresis, zymodem





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 CO-EXISTENCE OF BLASTOCYSTIS HOMINIS

 AND TRICHOMONAS HOMINIS IN AN IMMU PIGEON

 NOCOMPETENT PATIENT FROM NORTHERN
 IRAN
 Moh

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We report the co-existence of *B. hominis* and *Trichomonas hominis* in a 9-year-old immunocompetent boy suffering from chronic diarrhea and abdominal pain living in Northern Iran. Due to treatment with metronidazole (tid 750 mg for 5 days), the symptoms resorded and eradication of the organisms was observed. Our case shows that clinicians should be aware of both *B. hominis* and *T. hominis* as causes of chronic diarrhea in immunocompetent patients, particularly children. Such awareness will help in early diagnosis and avoid misdiagnosis with other gastrointestinal disorders.

Keywords: Blastocystis hominis, Trichomonas hominis, coexistence, Mazandaran

## PIGEONS TRICHOMONAS GALLINAE INFEC-TION RATE IN KHOY CITY, IRAN

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Pigeon breeding has been common for recreation and agriculture from a long time ago in most areas of Iran. Trichomonas gallinae infection is the most important problem of pigeon breeding, observed in all countries because of high intensity and crop milk feeding method of pigeon chicks. This survey aimed to investigate pigeons Trichomonas gallinae infection rate in Khoy city in 2014. One hundred and two pigeons were selected randomly from 4 areas of this city in May and June 2014. In order to examine the presence of Trichomonas gallinae, oral swabbing were done using warm normal saline impregnated swabs from oral cavity and pharynx and after preparation of smear and time/concentration calibration of the Giemsa stain, the smears were stained in the best process (1/12 concentration in 18 minutes) and microscopically examined under 400 and 1000 × magnifications. In overall, 5 (4.9%) out of 102 examined pigeons were infected to Trichomonas gallinae. All of 5 infected birds had oral cavity lesions and fetid mouth odor. Despite observed numerical difference, no statistical significant difference was observed about the prevalence rate in male and female birds.Despite drug interventions of the pigeon owners in Khoy, Trichomonas gallinae has a considerable prevalence that is important with respect to probable drug resistances due to arbitrary use of medicines and decreasing in pigeon breeding

Keywords: Trichomonas gallinae, Pigeon, Khoy





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PREVALENCE OF TRICHOMONAS VAGINALIS AND COMPARISON OF MOLECULAR WITH CULTURE METHODS AND INVESTIGATION OF WET MOUNT AND PAP SMEAR IN URMIA, IRAN

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Ineroduction & Objectives: Trichomonas vaginalis is a protozoan parasite that attack reproductive system and is one of the most common causes of non-viral sexually transmitted diseases. This parasite is found only in trophozoite form and its location is in vaginal environment or the prostate gland of the human beings. This parasite is produced nearly 180 million new infections annually across the world. This infection may result in urethritis and cervicitis in women. Preterm birth, low birth weight in babies and premature rupture of fetal membranes could be mentioned among other effects of this infection. The infection in men is often asymptomatic or sometimes appears with symptoms such as itching in ureter duct, secretion and irritation. Among 1825 of women referred to a diagnostic laboratory for control and Pap smears, vaginal sampling were taken and samples were analyzed using direct vision, culture, pap smear and PCR. Meanwhile a questionnaire contained items about personal and social status was completed and analyzed by SPSS statistical software. The amount of infection with trichomonas was obtained with mentioned methods as follow; direct vision 3.5%, culture 3.6%, Pap smear 3.3 %, respectively. PCR tests done on 74 detected positive samples that were detected using direct vision, culture or Pap smear and all samples were reported positive Trichomonas by PCR method. According to the results, the sensitivity of direct vision was 63.7%, culture 81%, Pap smear 61.7% and PCR, 100%. The specificity of four methods was 100%. Attention to the complications of the women infection by trichomoniasis, especially during pregnancy, early detection and treatment of the infection has the great importance.

Keywords: PCR, Tricomonas vaginalis, vaginitis, genotype

## THERAPEUTIC EFFECT OF ALLIUM CEPA, OL-IVERIA DECUMBENS VENT AND MUSCARI NE-GLECTUM ON TRICHOMONAS VAGINALIS, IN VITRO

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Trichomonas vaginalis is a flagellated pathogenic protozoa of human urogenital tract that infect 170 million new cases throughout the world annually. Considering the side effects of metronidazole as common drug to treatment of this disease, the present study was conducted to evaluate the in vitro activity of extract of Allium cepa, Oliveria decumbens vent and Muscari neglectum against Trichomonas vaginalis. This experimental study was carried out on Trichomonas vaginalis isolated from patients referred to five health centers in Kashan, Iran in 2013. The effect of alcoholic extract of Allium cepa, Oliveria decumbens vent and Muscari neglectum in 12.5, 25, 50, 100, 200, 400, 800and 1000 µg/ml concentration was evaluated on growth of Trichomonas vaginalis in TYI-s-33 medium after 12,24 and 48 hours. The results were compared with metronidazole effect in 0.025, 0.05, 0.1, 0.2, 0.4 µg/ml concentration as positive control. The number of alive and dead throphozoite measured and compared by trypan blue staininig. IC50 (Half maximal inhibitory concentration) was determined using GraphPad prism5. This study showed that the alcoholic extract of Allium cepa, Oliveria decumbens vent and Muscari neglectum had inhibitory effect on in vitro growth of Trichomonas vaginalis. Effect on growth of Trichomonas vaginalis in vitro condition. The IC50 rate was calculated 101.8 µg/ml for Oliveria decumbens Vent, 572.3 µg/ml for Allium cepa and 329.4 µg/ml for Muscari neglectum after 24 h. The IC50 rate of mrtonidazole was calculated  $0.0326\ \mu\text{g/ml}$  at the same time. Alcoholic extract of Allium cepa, Olivera dacumbens vent and Muscari neglectum has antitrichomonal effects. The effect of Oliveria decumbens vent was higher than others, we recommend further studies using major components of these plants especially Olivera dacumbens, to investigate its antitrichomoniasis effect.

Keywords: Trichomonas vaginalis, Allium cepa, Oliveria decumbens vent, Muscari neglectum, alcoholic extract





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Similar to other organs, heart is also affected by parasitic diseases. Myoccarditis, non-ischemic cardiomyopathies, pericarditis and increase in pulmonary artery blood pressure are among the most recognized complications of parasitic diseases. The most common parasitic disease affect the heart in glob especially in South America and tropical areas is Chagas disease, which is caused by Trypanosoma cruzi. 5-10 percent of the patients die because of acute myocarditis and 30-40 percent of infected cases affect by chronic cardiomyopathy. Echinococcosis is another common parasitic disease caused by different species of the genus Echinococcus. The parasite causes hydatid cyst in liver, lung and other visceral organs. Hydatid cyst is also forms in the myocardium, pericardium and heart cavities, which manifest as chest pain and arrhythmia. Many cases have been reported in Iran. Trichinellosis and taeniasis (due to Taenia solium) are other important parasitic diseases, which appear following pork consumption. Cysticercosis, the disease caused by larval stage of T. solium also affects human heart. About 25 percent of infected individuals manifest heart complications and myocarditis following infection by Trichinella spp. Some parasites, including Schistosoma and Entamoeba, which pass through circulation system, cause heart complications and increase in pulmonary artery blood pressure. In tropical regions, hypereosinophilia due to some parasitic infections cause endomyocardial fibrosis. Toxocara and Fasciola sometimes indicate such reactions.