

# 5<sup>th</sup> International and 12<sup>th</sup> National Congress of Parasitology & Parasitic Diseases of Iran (NICOPA XII)



## 5th International and 12th National Congress of Parasitology & Parasitic Diseases (NICOPA)

Congress Themes Challenges in: Clinical Symptoms, Diagnosis Methods and Treatment Approaches





21-23 May 2024



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## Message of the President

One of the most important factors in achieving a dynamic, healthy and active society is communicating and exchanging scientific information in the space of research as well as health development. For this reason, it is essential that all researchers and thinkers in the field of medical sciences realize the importance of the growth and development of health-related research. After holding several national congresses, we are proud to host a group of elites, researchers, doctors and students for three days at the 12th National Congress and the 5th International Congress of Parasitology and Parasitic Diseases in Iran.

In addition to welcoming the participants, first I consider it necessary to thank and appreciate the valuable support of the Parasitological Society of Iran for the magnificent holding of the Congress.

In the current congress, the latest and most recent scientific achievements and developments related to parasitic infections such as malaria, cutaneous *Leishmania*sis, Kala-azar, hydatid cyst, abortions caused by toxoplasmosis, visceral and ocular larva migrans, and emerging and reemerging parasitic diseases will be discussed.

It is hoped that due to the presence of prominent national and international scientist and researchers, the programs of the congress will be of interest to you and will be able to promote the science of medical parasitology and achieve the latest scientific findings in this field and cause scientific interaction and cooperation between researchers.

We are looking forward to your arrival at the International Congress of Parasitology and Parasitic Diseases in Karaj city, Alborz province, Iran.

## Dr. Shahram Sayyadi Congress President



## **ISP President**

The 12<sup>th</sup> National Congress and the 5<sup>th</sup> International Congress of Parasitic Diseases will be held in Iran with the efforts of the colleagues at Alborz University of Medical Sciences in 2024 by God's grace. The Iranian Society of Parasitology has always encouraged, supported these scientific congresses since the second conference until now, and has tried to make the results of scientific researches of these conferences useful for the country's health and parasitic disease control programs.

The importance of parasitic diseases in Iran is not hidden from anyone, and the historical evidence, especially in the case of diseases such as malaria and cutaneous *Leishmania*sis and infections caused by Helminthic parasites, is proof of this claim. The existence of important epidemics such as the outbreak of fascioliasis on two occasions in Guilan province and the spread of protozoan diseases such as *Leishmania*sis require that in order to prevent and discuss new scientific findings in these cases, from time to time experts and Practitioners of this field gather to share their knowledge to help the goals of fighting against parasitic diseases and spreading collective awareness. In this regard, the upcoming congress will be a unique opportunity for the dear practitioners of the field of parasitology to present their latest scientific achievements and help the dear students to step into a fruitful and bright future. It is obvious that the presence of foreign researchers will lead to more scientific fruitfulness of the congress and on the other hand, it will cause the interaction between professors and students related to different field of parasitology.

The Iranian Society of Parasitology considers it necessary to give special thanks in advance to the sincere efforts of each respected scientific and executive member of the Congress under the responsibility of the Department of Parasitology and Mycology of Alborz University of Medical Sciences and hereby declares its readiness to hold this conference as best as possible.

Prof. Hossein Keshavarz Chairperson Iranian Society of Parasitology (ISP)



## **Scientific and Executive Secretaries**

Dear colleagues

We are pleased to inform you that the 5th International Congress and the 12th National Congress of Parasitology and Parasitological Diseases in Iran jointly organized by the Iranian Parasitology Society (ISP) and Alborz University of Medical Sciences (ABZUMS) will be held on May 21-23, the main theme of which is "Advanced Innovations in Parasitology and Parasitic Diseases" which is organized with the aim of connecting scientists, researchers and clinicians.

In this congress, we plan to provide you and your organization with a wide networking opportunity with scientific, academic and international associations by holding meetings, seminars and training workshops with innovators and speakers. Our goal is to bring together outstanding academics from all over the country and the world so that the innovators of universities, laboratories and clinicians can meet with scientists and researchers in a scientific event and interact more closely with each other.

This fascinating event has taken an initiative to bring academic experts and research centers together on a common platform at the Congress of Parasitology and Parasitic Diseases to share their latest research findings with the world and bring respected scientists and researchers inform about the latest developments in this field.

We sincerely invite all interested people from all countries to join our event in Karaj, Iran and make it a success with their participation.

Prof. Mohammad Zibaei (Scientific secretary) Prof. Aliehsan Heidari (Executive secretary)

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# Oral & Poster Presentations

Oral Introduction of a candidate vaccine for cystic echinococcosis Abdolhossein Dalimi <sup>1</sup>© Ø, Sasan Khazaei <sup>1</sup>, Majid Pirestani <sup>1</sup>, Fatemeh Ghafarifar <sup>1</sup> <sup>1</sup> Department of Parasitology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

**Background:** Cystic echinococcosis (CE) is a neglected parasitic infection that has a significant health and economic impact on humans and livestock. The present study was conducted to introduce a candidate vaccine for CE.

Methods: At first, a multi-epitope vaccine was designed and evaluated in silico. Then it was transformed into E. coli TOP10 by recombinant pcDNA plasmid 3.1. Then the bacteria were mass-produced and the desired plasmid was extracted. BALB/c mice were immunized with concentrations of 50 and 100µg of plasmid together with IL-12 adjuvant or alone. Serum and spleen lymphocytes of mice were used to measure specific humoral and cellular responses. After immunization, the mice were challenged with live protoscoleces and finally the rate of growth of hydatid cyst in mice was investigated and the effectiveness of the vaccine was calculated.

**Results:** The mice receiving multi-epitope vaccine created higher levels of IgG2a and IFN- $\gamma$  antibodies in the mice, on the contrary, the mice showed lower levels of IL-4, which was statistically significant compared to the control groups. After the challenge, hydatid cysts were not observed in most of the vaccinated groups, while numerous cysts of different sizes were developed in the mice of the control group.

**Conclusion:** The absence of hydatid cyst formation or the presence of a small number of small cysts in vaccinated BALB/c mice highlighted the adequate immunogenic potential of this vaccine candidate.

Keywords: *Echinococcus granulosus*, vaccine, epitope, immunogenicity

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Oral

Diagnostic challenges in human toxocariasis: laboratory aspects

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Toxocariasis is a global cosmopolitan and typically neglected helminthic zoonosis. Larvae of Toxocara canis and T. cati primarily cause human infection. They are currently considered the main cause of visceral larva migrans (VLM), ocular larva migrans (OLM), covert toxocariasis (CT), and neurological toxocariasis. Due to different signs and symptoms including fever, pallor, malaise, irritability, weight loss, cutaneous rash, hepatomegaly, respiratory and nervous disturbs, myocarditis, hypergammaglobulinemia, leukocytosis and eosinophilia, visual loss, strabismus, retinal granuloma and detachment, endophthalmitis, chorioretinitis, uveitis; coughing, abdominal pain, headache, sleep and behavioral disturbances; the diagnosis of human toxocariasis usually can be difficult and may be misdiagnosed by different diseases. Diagnosis of the disease needs observation of clinical symptoms and paraclinical tests. Diagnosis may be missed due to insufficient awareness and complexity of clinical and laboratory diagnostic criteria. An IgG ELISA is a usual diagnostic method using native TES of T. canis. Using Western blot to confirm positive ELISA findings and reduce false-positive results is also suggested. Application of TES recombinant antigens and IgG4 subclass detection is used with success. Use of recombinant T. cati TES protein increases the diagnostic sensitivity. Development of antigen detection tests using polyclonal and monoclonal antibodies, can improve the antibody detection assays. A rapid test using a combination of rTES of T. canis and T. cati to achieve the diagnosis challenges especially in the regions endemic with both T. canis and T. cati infection is suggested.

Keywords: Toxocariasis, T. canis, T. cati, diagnosisCorrespondingAuthorsadjjadi316@gmail.com/zibaeim@sums.ac.ir



Anthelmintic resistance in livestock ruminants in Iran and worldwide: challenges and solutions

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There is a growing concern about anthelmintic resistance (AR) in farmed ruminants worldwide, and this is putting a strain on the effectiveness of parasite control. Research to combat AR will need to be intensified and integrated Innovative diagnostic tests need to be developed to detect helminth infections and AR, and sustainable antihelmintic treatments need to be developed, as well as complementary control methods like vaccination and plant-based methods. Additionally, in order to achieve a behavioral shift and to make sure the public adopts sustainable solutions, it will be necessary to gain a deeper understanding of socio-economic drivers of anthelmintic treatment decisions. The purpose of this lecture, is to review the state-of-the-art regarding AR in helminths of livestock ruminants in Iran and elsewhere. The spread of AR presents new challenges and solutions, and intensified research efforts demands transnational multi-actor initiatives to address these challenges and solve these problems. For infection control and sustainable control, all key stakeholders should be involved in the development of indicators and set targets, as well as promote good practices to accomplish them.

Keywords: Anthelmintic resistance, ruminants, challenges

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Oral

May 21 - 23, 2024

Misdiagnosis of cutaneous leishmaniasis

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Cutaneous Leishmaniasis (CL) has several clinical forms. However most of the clinical cases are presented & discussed by dermatologists and Parasitologists, but still we visit the patients who are misdiagnosed by clinicians & laboratory staffs. Misdiagnosis of cutaneous leishmaniasis may result to a chronic lesion, improper treatment, or even fake drug use. Therefore, the number of patients who are misdiagnosed by physicians and laboratory staffs is remarkable. Unusual clinical presentation, using improper, wrong or selftherapy, like using corticosteroids, fake treatment like using donkey machete, usually result to miss diagnosis of CL. In this session, we discuss about misdiagnosed cases and explain about the methods on real diagnosis of CL.

**Keywords**: Diagnosis, leishmaniasis, cutaneous

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Rapid diagnosis of cutaneous leishmaniosis using antibody conjugated gold nano particles in comparison with molecular and parasitological methods

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**Background**: Cutaneous leishmaniasis with a broad spectrum of clinical manifestations is caused by the *Leishmania* parasites and is transmitted by the bite of sand flies' insects. In parasitological methods, patients 'lesion biopsies either examined under microscope following appropriate staining or cultured in relevant medium. Although parasitological methods are very specific.

**Methods:** A lateral flow test for diagnosis of cutaneous leishmaniasis was developed in this work. For evaluation of the test, thirty-seven patient's samples with a clinical suggestion of CL were collected and examined in direct microscopy, culture, and PCR and the developed lateral flow test.

**Results**: Considering Microscopic examination, 28 out of 37 samples were positive and 9 out of 37 were negative. From 28 positive samples, 24, 26, and 28 became positive by cultivation, PCR and lateral flow test respectively. From negative samples, two were positive with lateral flow test. Considering microscopic results as gold standard, sensitivity of 92%, 92%, and 85% was estimated for lateral flow, molecular, and culture methods respectively. In addition, culture and molecular methods showed a specificity of 100%, however, with lateral flow specificity of 77% was detected.

**Conclusion:** Lateral flow test is a very raid easy using test for diagnosis cutaneous leishmaniasis especially in rural area with less facility.

Keywords: Cutaneous leishmaniasis, diagnosis, lateral flow

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Oral

10-year trends of epidemiology, diagnosis, and treatment of cutaneous leishmaniasis in Kohgiluyeh and Boyer-Ahmad province, south of Iran (2011–2021)

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**Background:** Cutaneous leishmaniasis is an arthropod borne disease that endemic in the south of Iran. Demographic changes in the last decade have changed the distribution pattern of this disease. This study was aimed to studying the frequency of CL in Kohgiluyeh and Boyer-Ahmad Province during 2011-2021 and its related demographic factors.

**Methods:** In this cross-sectional descriptive study carried out in Kohgiluyeh and Boyer-Ahmad Province (30.7246° N, 50.8456° E), information related to age, gender, occupation, place of residence, nationality, travel to endemic areas, number of lesions and its location, smear results, type of treatment and related complications were recorded using questionnaire. The data analyzed by descriptive and inferential statistics using SPSS 21.

**Results**: During the study years, 461 cases of CL including 275 cases (59.7%) in the rural areas and 186 cases (40.3%) in the urban regions reported in 310 male (67.2%) and 151 females (32.8%) with the mean age of  $29.34\pm18.8$  years old. 36.7% of the patients were from Kohgiluyeh county. 56.4% of CL lesions were dry. The most common site of lesions was in the face (27.3%). The highest frequency of CL was in 2014 (18.0% of all cases) and it had a relatively decreasing trend until 1400, so that the year 2021 included only 11.5% of all patients with CL.

**Conclusion:** There was a general decrease in the prevalence of CL in the province during the years 2014 to 2021. Although difficulties in the prevalence of CL were observed during the reviewed years, the overall trend of changes was downward.

Keywords: Cutaneous leishmaniasis, prevalence, Iran

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A clinical trial of the herbal combination of Zataria multiflora and Lawsonia inermis in the treatment of cutaneous leishmaniasis

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**Background**: Leishmaniasis is a parasitic disease that affects millions of people worldwide. Antimicrobial, anti-inflammatory and antioxidant properties of *Zataria multiflora* and *Lawsonia inermis* are known. This study was conducted with the aim of investigating the anti-Leishmanial activity of the herbal combination of *Z. multiflora* and *L. inermis* on patients with leishmaniasis.

**Methods:** This study was conducted in Kherameh city of Fars province. The criteria for entering patients into this study included individual characteristics (age, sex, and occupation) and clinical (number of wounds and period of the wound). The patients were divided into two groups, the first group received Glucantime drug and the second group received the herbal drug considered in this study (*Z. multiflora- L. inermis*). The number of participating patients was 40 in the first group and 90 in the second group. Then the patients were followed up at intervals of 14, 45 and 60 days after the start of treatment.

**Results**: After 14 days from the start of treatment, 38 people who used the herbal medicine and 5 people who used the glucantime medicine recovered. After 45 days, 36 people who took the herbal medicine were completely cured, and only 5 people who received the glucantim medicine were cured. Finally, after 60 days, six of the people who took the herbal medicine recovered completely, but none of the people who took Glucantim recovered. Finally, 71.2% of the people who received herbal medicine and 17.9% of the people who received glucantime recovered completely.

**Conclusion:** The results of this study showed that the herbal combination of *Z. multiflora* and *L. inermis* has equal effectiveness compared to the first-line drug (glucantime) on patients with cutaneous leishmaniasis. Further research is needed to evaluate its efficacy for potential use as a new treatment option for leishmaniasis in humans.

Keywords: Leishmaniasis, Z. multiflora, L. inermis

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Oral

A new sensitive chemical based fecal occult blood test without false positive results

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**Background**: Nowadays chemical-based (gFOB) and immunological-based (FIT) methods are used for diagnosis of fecal occult blood in the world. In chemical-based tests, the hem part of hemoglobin reacts with a chromogenic material in the presence of hydrogen peroxidase. Therefore, in patients eating fruits and vegetables, false positive results may occur.

**Methods:** For estimation of analytical sensitivity serial dilution of the blood was prepared in distilled water and then they were tested. For investigating false positive results, different fruits and vegetables such as radish and orange were tested with these methods and other fecal occult blood kits, which were available in the market.

**Results**: From analytical point of view, the developed test is able to detect 60 nanogram or more hemoglobin in one-milliliters of water. Results of the test with different fruits and vegetables showed that while chemical based fecal occult blood kits had false positive results; the test developed in this work has no false positive results with fruits and vegetables.

**Conclusion:** The test developed in this work has a high sensitivity without any false positive results.

Keywords: Fecal occult blood, sensitivity, false

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Ace-1 target site status associated with bendiocarb resistance in the field populations of main malaria vector, *Anopheles stephensi* in Iran

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**Background**: Anopheles stephensi is the main vector of malaria in Iran. This study aimed to determine the susceptibility of An. stephensi from the south of Iran to bendiocarb and to investigate molecular resistance mechanisms in this species.

**Methods:** Wild *An. stephensi* were collected from Hormozgan Province and reared to the adult stage. The susceptibility test was conducted according to the WHO protocols using bendiocarb impregnated papers supplied by WHO. Also, field *An. stephensi* specimens were collected from south of Kerman and Sistan and Baluchistan Provinces. To determine the G119S mutation in the acetylcholinesterase (Ace1) gene, PCR-RFLP using AluI restriction enzyme and PCR direct sequencing were performed for the three field populations and compared with the available GenBank data.

**Results**: The bioassay tests showed that the *An. stephensi* field strain was resistant to bendiocarb (mortality rate 89%). Ace1 gene analysis revealed no G119S in the three field populations. Blast search of sequences revealed 98–99% identity with the Ace1 gene from Pakistan and India respectively.

**Conclusion:** The use of alternative malaria control methods and the implementation of resistance management strategies are suggested in the study area.

Keywords: Anopheles stephensi, malaria, insecticide resistance

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#### Oral

Application of different strategies of CRISPR/Cas9 technology for genome editing in *Leishmania* parasites

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**Background**: To date, different approaches of genome editing by CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated gene) system has been applied to *Leishmania* parasites. In *L. major* and *L. donovani* a 2-vector strategy was employed to target different copies of PFR2 tandem and miltefosine transporter genes, with successful KO generation.

**Methods:** Here, we report establishment of a CRISPR/Cas9 method adapted from Eva Gluenz et al., which comes as a toolkit, protocols for both gene deletion and N- or C-terminal tagging, as well as a website for designing the sgRNA primers and the donor DNA cassette are provided (LeishGEdit). We applied the method to different genes including centrin and a kinetoplast gene for tagging and KO generation. *L. major* cell line with an episomal plasmid expressing Cas9 and T7RNA polymerase was produced, then PCR-amplified sgRNA template and donor DNA repairment cassette with resistant marker were transfected to *L. major* cell line.

**Results**: Using this fast and accurate genome editing, successful C-terminal tagged kinetoplast gene and monoallelic and biallelic mutants of centrin gene deleted *Leishmania* parasites have been produced. We found this method more flexible than other approaches, which were developed earlier.

**Conclusion:** There is a progressive propensity to use different types of CRISPR/Cas9 for gene editing and tagging to elucidate gene functions; one of which has introduced as an easy-to-use technique without need of cloning for *Leishmania* editing.

Keywords: Leishmania, CRISPR/Cas9, genome editing

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Assessment of the therapeutic effect of spiramycin nanoemulsion on acute and chronic toxoplasmosis in mice

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**Background**: *Toxoplasma* infection is caused by *Toxoplasma gondii*, an intracellular protozoan parasite. Spiramycin is typically recommended for *T. gondii* infection in pregnant women. The present study aimed to prepare the nanoemulsion of spiramycin (NE-Spi) and to evaluate its activity in the treatment of acute and chronic toxoplasmosis in mouse models.

**Methods:** NE-Spi was prepared using spontaneous emulsification by soybean oil as the oil phase; a mixture of Tween 80 and 85 as a surfactant; ethanol as a co-surfactant and distilled water. Particle size and zeta potential of NE were assessed. The stability testing of NE was assessed after storage for 1 month at room temperature and three freeze–thaw cycles. In vivo experiments were carried out using 40 BALB/c mice inoculated with virulent RH strain and 40 BALB/c mice inoculated with a virulent Tehran strain of *Toxoplasma gondii* and treated with NE-Spi, Spi suspension, and NE without Spi.

**Results**: The final particle size and zeta potential of the synthesized NE-Spi were calculated to be 11.3 nm and  $-33.7\pm2.45$  mV, respectively. In acute toxoplasmosis, the mice treated with NE-Spi showed a significant reduction in the tachyzoite count of the peritoneal cavity and a longer survival time. The differences were statistically significant between the survival times and tachyzoites count of mice in the NE-Spi treated group compared with the negative control group. In the chronic phase, NE-Spi administration significantly decreased the mean number and the average size of the brain cysts of chronically infected mice with *T. gondii*, Tehran strain.

**Conclusion:** Results from the current study showed Oral administration of NE-Spi increased mice survival time and reduced the number of tachyzoites, and the number and size of brain cysts.

Keywords: Spiramycin, nanoemulsion, toxoplasmosis, Tehran strain

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Oral

Cutaneous leishmaniasis retards COVID-19: A large-scale case-control study in major endemic foci of southeastern Iran

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**Background**: We assessed the potential relationship between COVID-19 and laboratory-confirmed cutaneous leishmaniasis (CL)-registered cases with a history of scarring, compared with volunteer participants without a history of CL. We hypothesized that the immunological memory cells generated by some infectious diseases, such as CL, could reduce the incidence and severity of COVID-19.

**Methods:** This case-control study was performed between July 2020 and December 2022 in the high-burden endemic areas of CL in the southeast of Iran. In the study, 1400 previous CL cases with scars and 1,521,329 subjects who had no previous CL were analyzed. We used R 4.0.2 to analyze the data. Firth's bias reduction approach corresponding to the penalization of likelihood logistic regression by Jeffreys was also employed to influence the variables in the dataset. In addition, a Bayesian ordinal logistic regression model was performed to explore the COVID-19 severity in both case and referent groups.

**Results**: In general, in the CL scar group, 1400 laboratoryestablished patients with previous CL scar were analyzed. In the control group, 117,185 subjects (7.70%) with contracted laboratory-confirmed COVID-19 infection were assessed. The occurrence and severity rate of COVID-19 in CL scar cases are significantly less than in the non-CL control group, while in the CL scar subjects, patients with critical conditions and mortality were not observed. The morbidity (OR = 0.11, CI = 0.06–0.20 and P = 0.001) and severity of COVID-19 in previous cases with CL scars were significantly diminished relative to the control group (credible interval – 2.57, – 1.62).

**Conclusion:** The results showed a durable negative relationship between healed CL and COVID-19 incidence and severity. Perhaps there is a cross-protective effect against SARS-CoV-2 infection. More studies seem needed to validate further the true effect and underlying mechanistic action of CL against COVID-19.

Keywords: Cutaneous leishmaniasis, retards, COVID-19, Iran

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Deciphering the role of miR-71 and let-7 in the fertility of cystic echinococcosis: a preliminary assessment.

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**Background**: Cystic echinococcosis (CE) is a neglected helminthic zoonosis. Some CE cysts in the intermediate host are non-fertile. Considering the function of microRNAs in many biological processes such as embryonic development, cell proliferation, and apoptosis, this study investigated the function and comparison of miR-71 and let-7 in fertile and non-fertile CE

**Methods:** Here, we determined the expression level of the miRNAs for 33 animal cysts and 16 human cysts (*E. granulosus* sensu stricto (G1). Total RNA was extracted from cyst wall samples using the RNX-Plus Mini Kit according to the manufacturer's protocol. Then, cDNA was synthesized using a stem-loop system. Expression evaluation of miR-71 and let-7 was conducted by the qRT-PCR method. The expression of both miRNAs in all samples was determined using the following formula: [ $\Delta CT = CT$  (target) – CT (internal control)].

**Results**: A comparison of  $\Delta$ ct of miR-71 and let-7 in fertile and non-fertile cysts did not show a significant difference (P = 0.911 and 0.354). In cattle, sheep, and humans,  $\Delta$ ct of miR-71, and let-7 were higher, respectively. Therefore, the mean expression of miR-71 and let-7 indicates an increase in humans compared to other intermediate hosts. In addition, statistical results show a significant difference in the expression of these miRNAs in sheep, cattle, and human cysts (P = 0.025 and 0.01).

**Conclusion:** The lower expression of these miRNAs in cattle cysts and their common infertility might be associated with function of miRNAs in the fertility of CE cysts. Therefore, we should not ignore the function and role of miRNAs in this subject due to the importance of infertility in *E. granulosus* epidemiology.

Keywords: Hydatid cyst, micro RNAs, miR-71,

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Oral

Designing of Multiplex Microfluidic LAMP PCR for simultaneous detection of gastrointestinal protozoa including *Giardia lamblia, Cryptosporidium* spp., and *Enterocytozoon bieneusi* 

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Background: Three protozoa Giardia lamblia, Cryptosporidium, and Enterocytozoon bieneusi are of great diagnostic importance. The LAMP method is performed in a constant temperature with high sensitivity. Microfluidic technology uses small devices that is used as point-of-care diagnostic (PoC) tests for clinical patients. Methods: 30 positive samples consisted of 30, 19, and 30 G. lamblia, Cryptosporidium, and E. bieneusi, were included in this study. Positive samples were examined by real-time PCR and LAMP method. The primer specificity was evaluated using couple of microorganisms. The primer sensitivity was evaluated using six serial dilutions of log-10 of DNA. The microfluidic disc was designed using AutoCAD software and fabricated by laser beam. The multiplex accuracy of the MµLAMP PCR was determined using the two-parasite and three-parasite DNA mixes. Results: The real-time analysis showed that 27/30 (90 %), 18/19 (94.7 %), and 30/30 (100 %) of G. lamblia, Cryptosporidium spp., and E. bieneusi samples, respectively, were positive. The LAMPanalysis revealed that 29/30 (96.7 %), 12/19 (63.2 %), and 25/30 (83.3 %) samples were positive for G. lamblia, Cryptosporidium, and E. bieneusi, respectively. The specificity-results showed no false-positive reactions with other microorganism. The sensitivity results indicated both LAMP and MµLAMP detect concentration of DNA up to 18 fg/µl of G. lamblia, 25 fg/µl of Cryptosporidium, and 34 fg/µL of E. bieneusi. The microfluidic-disc showed that

primers cause false-positive results. **Conclusion:** The results of the present study indicate that the MµLAMP PCR method is a rapid, sensitive and highly specific method for the simultaneous detection of the protozoa, making it a suitable tool for screening and PoC testing.

none of two-and three-parasite DNA-mixes with their non-specific

**Keywords**: Intestinal parasites, LAMP, multiplex-microfluid-LAMP, point-of-Care

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#### Oral

Detection of *Cryptosporidium* spp. directly in clinical specimens by molecular and microscopic methods in HIVpositive patients

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**Background**: Despite the huge advance of antiretroviral therapy represent for HIV, opportunistic infections continue to be a cause of morbidity and mortality in HIV-infected patients. *Cryptosporidium* species are commonly known to cause chronic intractable diarrhea and life-threatening disease in HIV-positive patients; it can also infect the biliary system and respiratory tract.

**Methods:** The study was conducted on 100 HIV-positive patients suspected of having opportunistic infections. Clinical samples including nasopharyngeal swabs, induced sputum and stool specimens were collected from each patient and routine clinical evaluations were performed. Microscopic examination (modified acid-fast and modified trichrome staining) was applied on sputum and stool specimens. Detection and species identification were done using nested-PCR-RFLP. Data analysis was performed utilizing SPSS statistical software version 19 and analysis of variance (ANOVA).

**Results**: Among the 100 patients, *Cryptosporidium* infections were detected in 9 patients (9 stool, 3 sputum, and 3 NP), including 5 cases of *C. hominis* and 4 cases of *C. parvum*. There was a significant relationship between age, CD4 count, transmission route of *Cryptosporidium*, level of education, and adherence to treatment with opportunistic infections incidence.

**Conclusion:** HIV-positive patients with a CD4 count less than 200 cells/ $\mu$ L are more vulnerable to opportunistic infections. Early isolation, identification, appropriate treatment and routine screening of opportunistic pathogens can reduce mortality due to co-infections. Molecular methods demonstrate superior sensitivity for detection of *Cryptosporidium* spp. compared to microscopic methods.

Keywords: HIV, Cryptosporidium, gastroenteritis, RTIs

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#### Oral

Detection of *Dientamoeba fragilis* DNA in scotch tape samples containing *Enterobius vermicularis* eggs using small-subunit rRNA (SSU rRNA) gene by PCR method

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**Background**: *Dientamoeba fragilis* is a protozoan with unknown mode of transmission due to no known cyst stage. A hypothesis is that the *Enterobius vermicularis* eggs could serve as the vector for D. fragilis. This study aimed to detect *D. fragilis* DNA in scotch tape containing *E. vermicularis* eggs using molecular method.

**Methods:** A total of 34 scotch tape samples containing *E. vermicularis* eggs were collected. Genomic DNA was extracted from 34 scotch tape samples containing *E. vermicularis* eggs using a commercial kit. The small-subunit rRNA (SSU rRNA) region was amplified by the polymerase chain reaction (PCR) method and sequenced. The obtained sequences were aligned using the BioEdit software and compared with the published sequences in GenBank using BLAST system. Phylogenetic analysis was performed using MEGA 5.0 software.

**Results**: The molecular method showed that 14 out of the 34 scotch tape samples containing *E. vermicularis* eggs were infected with *D. fragilis* (41.2%). BLAST analysis indicated that the sequenced isolates belonged to *D. fragilis* genotype 1.

**Conclusion:** Detection of *D. fragilis* from *E. vermicularis* eggs needs cloning and observation of *D. fragilis* in eggs, which is so hard work. However, the detection of *D. fragilis* DNA in scotch tape containing *E. vermicularis* eggs can help to interpret the hypothesis of *D. fragilis* transmission by *E. vermicularis* eggs.

**Keywords**: *Dientamoeba fragilis*, scotch-tape, *Enterobius vermicularis*, eggs, SSU-rRNA

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Development and optimizing a simple and cost-effective medium for in vitro culture of *Plasmodium berghei*-ANKA strain with conserving its infectivity in BALB/c mice

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**Background**: The current culture system for *P. berghei* still requires modifications in consistency and long-term maintenance of parasites considering their pathogenicity in culture media. This study designed to further improvement of culture conditions and designing a cost-effective culture medium with minimum changes in pathogenicity for in vitro culture of *P. berghei*.

**Methods:** Male Bulb/C mice were infected via inoculation of infected RBC suspension intra-peritoneally. When parasitaemia reached more than 5%, blood samples from infected mice were collected. All blood samples underwent the buffy coat removal process using routine PBS washing method and cellulose column then culturing was conducted in small petri-dishes, storing at 34°C in candle jar. Composition of our medium was RPMI1640, FCS, NaOH, Gentamicin, Albumax II, infected and non-infected mice red blood cell. In addition, In vivo infectivity test of the cultured parasites was evaluated by inoculating IRBCs in susceptible mice (BALB/c) using IP inoculation.

**Results**: Results indicated that the rate of parasitaemia in our method remained statistically stable between days one to seven. The current modified cultivation method was more efficient in maintaining of parasites for further days. Furthermore, the virulence of cultivated parasites in our modified method remained similar to frozen stock parasites as positive control group. No significant differences were seen in survival time between two groups of mice those were infected with either cultivated parasites or stock freeze parasites

**Conclusion:** In vitro Life cycle stages of the *Plasmodium* species have been established earlier. However, different degrees of success have been achieved with the *Plasmodium berghei* because of sensitive and low-stability nature of this parasite. In this study, we employed simple method for *P. berghei* cultivation without previous complicated and expensive methods

Keywords: Malaria, *Plasmodium berghei*, in vitro

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Oral

Effect carbohydrates of laminitis layer, liquid and protoscolex hydatid cyst on the human colorectal adenocarcinoma cell line (LS174T).

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**Background**: Hydatid disease or hydatidosis is a common disease between humans and animals caused by the larval stage of *Echinococcus granulosus*. Cancer is a genetic or epigenetic disorder of somatic cells that is associated with abnormal cell growth and the ability to invade other body tissues.

**Methods:** In this study, livers infected with hydatid cysts were collected, and their glycolipids and glycoproteins were separated using the chloroform-methanol method. Sugars were precipitated by beta-elimination method, impurities were separated using dialysis bag and different fractions were prepared by ion exchange chromatography. After redialysis using the phenol sulfuric acid method, carbohydrates were precipitated and absorbance was measured using an ELISA reader. Six fractions from each part of the cyst were separated by ion-exchange chromatography, resulting in 18 fractions. Each of the fractions was directly entered into the dialysis bag, and after 24 h, the carbohydrates were separated.

**Results**: The results of the present study showed that the fraction of 0.125 and the fraction of 0.25 glycolipid in the laminitis layer (cow) significantly inhibited the growth of cells, and the reduction in cell growth was significant compared to that of doxorubicin.

**Conclusion:** The results of our study are consistent with those of previous studies that showed that carbohydrates isolated from different parts of hydatid cysts have significant toxic effects on the colorectal cancer cell line LS174T and on other cancers, including breast cancer.

Keywords: Hydatid cyst, doxorubicin, carbohydrate, cancer,

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Evaluation of a newly designed chimeric recombinant antigen (SsIR-Ss1a) for the serodiagnosis of human strongyloidiasis: an assessment of efficacy, sensitivity, and specificity

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**Background:** Parasitological and molecular methods have low sensitivity for diagnosing strongyloidiasis, and serological approaches remain the most effective diagnostic strategy. The current study employed immune-informatics methodologies to construct and generate a chimeric recombinant antigen from *Strongyloides stercoralis* immunoreactive antigen (SsIR) and Ss1a antigens, which was then tested in an ELISA system.

**Methods:** The coding sequences for SsIR and Ss1a were taken from GenBank and gene-optimized. Bioinformatics analysis was used to select the areas with the highest immunogenicity that did not overlap with other parasite antigens. A chimeric recombinant antigen, SsIR-Ss1a, was constructed. The construct was expressed in the pET-23a (+) vector, and the optimized DNA sequences of SsIR-Ss1a (873 bp) were cloned into competent *E. coli* DH5 $\alpha$  cells. The diagnostic efficacy of the generated recombinant antigen, accompanied by a commercial kit, was tested in an ELISA system with a panel of sera from strongyloidiasis patients and controls.

**Results**: The physicochemical and bioinformatics assessments indicated that the antigenic and soluble chimeric construct had a molecular weight of 35 kDa. Western blotting verified the generated chimeric recombinant antigen's immunoreactivity with serum from strongyloidiasis patients. The ELISA system's sensitivity and specificity, using the generated SsIR-Ss1a chimeric antigen, were determined to be 93.94% (95% CI, 0.803-0.989) and 97.22% (95% CI, 0.921-0.992), respectively.

**Conclusion:** The study's findings showed that the developed SsIR-Ss1a chimeric antigen is reliable in the diagnosis of human strongyloidiasis. This construct could potentially serve as a useful antigen in an ELISA system for the serological diagnosis of this neglected parasitic infection.

Keywords: Strongyloidiasis, recombinant chimeric antigen, bioinformatics

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Oral

Evaluation of *Fasciola* Excretory/Secretory (E/S) antigen for serodiagnosis of fascioliasis in a military unit

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**Background**: Fascioliasis is an importance zoonotic disease commonly transmitted by herbs or vegetables infected with metacercariae of the parasite. Excretory/Secretory antigen of *Fasciola* spp. has shown acceptable diagnostic accuracy. The current study aimed to providing and evaluation of *Fasciola hepatica* (E/S) antigen for serodiagnosis of human fascioliasis in a military unit.

**Methods:** After the production of *Fasciola* E/S antigen, its efficacy was compared with a commercial kit designed for the detection of fascioliasis. One hundred and fifty seven serum samples, including 41 positive sera from confirmed infected patients, 41 sera from people suffering from non-relative parasitic infections, and 75 sera from healthy people were utilized for evaluating. In second stage, 205 samples were evaluated using *Fasciola* E/S antigen by ELISA method for serodiagnosis of fascioliasis in a military unit.

**Results**: Sensitivity, specificity and accuracy of Elisa using *Fasciola* E/S antigen were 97.56%, 98.28% and 98.09%, respectively. The values of the commercial kit were 95.12%, 97.41% and 96.82%, respectively. Twenty-two of 205 (10.7%) samples from military personnel have positive response to E/S antigen of *Fasciola*.

**Conclusion:** Considering the reliability, accuracy and acceptable characteristics of the produced E/S antigen in compare with commercial kit, it can be used in the serodiagnosis of fascioliasis in endemic regions. Seroprevalence of fascioliasis in military personnel is significant; therefore, the necessary notification should be made in the prevention and control purposes.

Keywords: Fasciola, Excretory/Secretory (E/S) antigen, serodiagnosis

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Evaluation of genes expression in hepatic fibrosis caused by human hydatidosis

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**Background**: Hepatic fibrosis is a dynamic process that occurs in response to chronic liver diseases resulted by various factors, multicellular parasites such as *E. granulosus* cause chronic inflammation and the development of liver fibrosis. The aim of this study was to assess gene expression in liver fibrosis associated with cystic echinococcosis.

Methods: The fibrotic and healthy liver tissue of 25 CE patients have been investigated. In order to assess the pathological morphology of fibrotic and healthy liver tissue including deposition of fat, inflammation, and congestion of RBCs with Hematoxylin and Eosin (H&E) staining and for collagen deposition and fibrosis with Masson's Trichrome (MT) staining were used to investigate the development of hepatic fibrosis. The expression of ACTA2, COL3A1, IFN-y, MMP2, MMP9, Smad2, Smad3, TGF- $\beta$ 1, and TNF- $\alpha$  genes was determined by qRT-PCR in healthy and fibrotic liver tissue of the 25 CE patients. Furthermore, TGF-β1 evaluated expression was by Immunohistochemistry (IHC) staining.

**Results**: Expression of MMP9, ACTA2, COL3A1, and MMP2 was found significantly higher in the fibrotic tissue compared to healthy. Although expression of the Smad2 gene was higher in the fibrotic tissue, difference from healthy tissue was not significant. The mRNA levels of IFN- $\gamma$  and Smad3 were lower in the fibrotic than in the healthy hepatic tissue. Immunohistochemistry analysis revealed TGF- $\beta$ 1 up-regulation in the fibrotic tissue. Histology showed inflammation and fibrosis to be significantly higher in the fibrotic tissue.

**Conclusion:** The findings suggest that *E. granulosus* can promotes fibrosis through overexpression of TGF- $\beta$ 1, MMP9, ACTA2, COL3A1, and MMP2. The downregulation of IFN- $\gamma$  mRNA in fibrotic samples is probably due to the increased production of TGF- $\beta$ 1 and the suppression of potential anti-fibrotic role of IFN- $\gamma$  during advanced liver injury caused by *E. granulosus*.

**Keywords**: Liver fibrosis, cystic echinococcosis, TGF-β1 **Correspondence Email(s):** beiromvandm@gmail.com Oral

Evaluation of microscopic method and rapid diagnostic test compared to polymerase chain reaction test in the detection of *Plasmodium vivax* parasite in suspected malaria cases

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**Background**: This study was carried out with the aim of evaluating the rapid diagnostic test (RDT) and microscopic method in comparison with PCR in the detection of *Plasmodium vivax* parasites in suspected malaria cases.

**Methods:** In a cross-sectional study, out of 207 febrile patients suspected of malaria, for microscopic diagnosis, blood sample was taken from each person's finger and spread, and after staining with Giemsa dye, were examined with a 100 X optical microscope lens. Whole blood samples were collected from all suspected individuals and rapid diagnostic test (RDT), and DNA extraction and PCR was performed. To check the validity of different methods while calculating sensitivity, specificity, positive predictive value, negative predictive value, drawing ROC curve, Kappa coefficient ( $\kappa$ ) was used to measure the agreement of the tests.

**Results**: In this study, microscopy and RDT compared to PCR have sensitivity of 96% and 94% and specificity of 100% and 98.2%, respectively the kappa coefficient in the microscopic method is0.96 and the RDT was 0.91.

**Conclusion:** Considering the values of sensitivity and specificity, as well as the high kappa coefficient in the microscopic method and RDT, both methods have a very good agreement with the PCR.

Keywords: Malaria; PCR, RDT, Plasmodium vivax

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Evaluation of the frequency of suicide in the patients with chronic phase of *Toxoplasma gondii* infection

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**Background**: *Toxoplasma gondii* is an obligate intracellular protozoan that in the host have an affinity to the brain (microglial) and can become a cyst. Many epidemiological studies shown that chronic toxoplasmosis is associated with an increased likelihood of neuropsychiatric disorders such as schizophrenia, bipolar disorder, epilepsy, and Alzheimer's disease.

**Methods:** In this study, 177 people with schizophrenia and bipolar disorder participated, who were examined for the serum prevalence of specific anti-*Toxoplasma* antibody (IgG) using ELISA and a questioner contain suicide history were complete. They were analyzed using statistical tests and logistic regression. Considering the 95% confidence interval and P < 0.05, it was considered statistically significant.

**Results**: In the present study, 9.6% of people in the patient group attempted suicide in general (17 patients). About 88% of them were toxoplasmosis positive, which showed a significant relationship between suicide and toxoplasmosis compared to the toxoplasmosis negative group. In addition, the study showed that people who committed suicide had higher IgG antibody titers compared to people who did not commit suicide.

**Conclusion:** *T. gondii* could be one of the biology cause of suicide or tendency to suicide in patients with Psychological disorders.

Keywords: *Toxoplasma gondii*, suicide, schizophrenia, bipolar

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Oral

Exploring the significant genetic diversity of Iranian isolates of *Leishmania* RNA virus 2 using whole genome sequence analysis

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**Background**: Our work presents the whole genome sequence and phylogenetic analysis of five *Leishmania* RNA virus 2 (LRV2) isolates obtained from patients with cutaneous leishmaniasis (CL) in Iran.

**Methods:** The total RNA from  $1-5 \times 106$  promastigotes in the stationary phase was isolated following the instructions provided by the manufacturer (Favorgen RNA extraction kit, Favorgen Biotech). The cDNA synthesis was carried out using a combination of oligo (dT) 18 and random hexamer primers in accordance with the instructions provided by the cDNA Synthesis Kit (YTA, Iran with Cat No: YT4500). To perform whole genome sequencing of the LRVs, ten pairs of primers were designed using Oligo 7 primer design software.

**Results**: The current sequences were most closely related to the whole genome of LRV2 previously reported from Uzbekistan and Turkmenistan. The whole genome sequence of the LRV2 showed high genetic and haplotype diversity. The study also revealed the existence of three distinct clades of LRV2, with the LRV2 sequences infecting *L. major, L. aethiopica*, and sauro *Leishmania* belonging to separate lineages. These lineages have seemingly evolved independently, as the geographic distribution of their flagellate hosts (Ethiopia, Senegal, and Kenya) does not overlap with the *Leishmania* species. The divergence between these three clades is attributed to considerable antiquity, leading to genetic modifications within

**Conclusion:** These findings contribute to our understanding of the genetic diversity and evolution of LRVs, providing valuable insights into their role in *Leishmania* infections. Further investigations are needed to understand the significance of these polymorphic sites and their potential impact on viral characteristics and disease outcomes.

**Keywords**: Cutaneous leishmaniasis, genetic diversity, *Leishmania* 

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Oral

Genetic variation in genes of *Plasmodium vivax*, the dominant malaria species in Iran

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**Background**: Malaria is an important disease worldwide. The disease is considered low endemic in Iran, with *Plasmodium vivax* being responsible for over 90% of reported cases. A fundamental step towards developing malaria vaccine involves identifying haplotypes and determining the genetic diversity of genes that encode immunogenic proteins in *P. vivax*.

**Methods:** Totally 40 malaria patients with clinical manifestation participated in this study that conducted in Sistan and Baluchistan, Iran in 2023. Genetic diversity were studded in two genes responsible for coding surface antigens of *P. vivax* blood merozoites including Apical Membrane Antigen 1 (AMA1) and Merozoite surface Protein 5 (MSP5). Two mL blood were taken from the each patents. DNA was extracted using the FavorPrep Blood Genomic DNA Extraction Mini Kit. The PCR products of two mentioned genes of *P. vivax* isolates were sequenced by Bioneer Company in South Korea.

**Results**: All isolates were *P. vivax* using nested PCR and ribosomal ribonucleic genes of *Plasmodium* 18 subunit ribosomal ribonucleic (Ssr RNA) genes. Using ClustalW, DNASP, MEGA software revealed the genetic diversity of the sequenced isolates. The phylogenetic trees of nucleotide sequences corresponding to the studied isolates, and the sequences received from GenBank were showed that the Iranian *P. vivax* isolates were new haplotypes and were different from the recorded sequenced in GenBank.

**Conclusion:** the study of two genes coding antigens of *P. vivax* blood merozoites, it has been determined that the endemic region exhibits a high level of genetic diversity. The influence of factors including traffic and migration from neighboring countries, natural selection and recombinant events played an important role in polymorphism.

Keywords: *Plasmodium vivax*, polymorphism, malaria, AMA1, MSP5

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#### Oral

Frequency survey of *Pneumocystis jirovecii* and *Microsporidia* spp. in bronchoalveolar lavage (BAL) specimens of immunocompromised patients with pulmonary symptoms in selected hospitals in Tehran in 2022 – 2023

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**Background**: Pneumocystosis and pulmonary microsporidiosis are two pulmonary infections; the causative agents of these two infections are one fungal and the other protozoan. Considering the many issues for diagnosis in health and treatment centers, this study intends to investigate the presence of *Pneumocystis jirovecii* and microsporidia in suspected patients.

**Methods:** A total of 76 BAL samples were gathered by a specialist doctor including 49 samples from immunodeficiency patients with pulmonary symptoms and 27 samples as control base, sent to the laboratory in a sterile condition. Giemsa staining was used for *Pneumocystis jirovecii* whereas Ziehl-Neelsen staining and chromotrope 2R were used for microsporidia. The molecular test was done by Nested-PCR and Real-time PCR methods.

**Results**: Out of 49 patients, Giemsa staining for *P. jirovecii* reported only 2 (4.08 %) patients positive. Among 49 patients, no one was reported positive for *Microsporidia* by Nelson's substaining and Chromotrope 2R. For 49 samples of immunodeficiency patients, Nested-PCR results for *P. jirovecii* were positive for 9 (18.36 %) patients. The DNA samples of these 9 patients were also checked with Real-time PCR, and 6 (12.24 %) cases were reported positive. From the 27 samples of the control group, the Nested-PCR result of *P. jirovecii* was only positive for one patient.

**Conclusion:** Recognition of *P. jirovisii* by microscopy has been difficult due to the low sensitivity of conventional staining methods such as Giemsa staining. Molecular methods can be considered useful for diagnosing unrecognized infections. The high sensitivity of molecular methods may be related to the detection of trophozoite forms of the organism.

Keywords: *Pneumocystis jirovsii*, Microsporidia, BAL samples, immunodeficiency Correspondence Email(s): samansari1988@yahoo.com



Oral

Genotyping and phylogenetic study of *Giardia lamblia* at northeast of Iran

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**Background**: *Giardia lamblia* is a common intestinal protozoan of human, which has a highly diverse genetic assemblage. Since clinical pictures and pathogenesis of the parasite vary in different individuals, genetic assemblage may be responsible. The objective of this study was to determine assemblage and sub assemblage of *G. lamblia* inhuman.

**Methods:** In this cross sectional study, 13 *G. lamblia* samples, isolated directly from stool of symptomatic and asymptomatic patients were analyzed. PCR assay was done based on DNA sequences of B-giardin gene. Neighbor joining (NJ) analysis and genetic distance within and between assemblage was assessed using MEGA X software. In addition, a phylogenetic analysis was carried out based on the DNA sequences obtained from these *Giardia* and those available in GenBank.

**Results**: Three A assemblages (Sub-assemblages A2 and A3) and 10 B assemblages (Sub-assemblages B2 and B3) were obtained. Two of 15 B-giardin gene sequences remained unclear. Nine patients with clinical signs were detected to have B assemblage (P = 0.003).

**Conclusion:** Although, the sample size of this study was limited, it can be concluded that genotype B is directly related to clinical symptoms and this genotype is probably more widespread in Khorasan-e-Razavi and northeastern Iran. Further studies covering greater population is recommended.

Keywords: Genotyping, phylogeny, Giardia lamblia

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Oral

Hepatozoonosis in cats in Iran

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**Background**: Although infections of cats with *Hepatozoon felis*, *H. silvestris* and *H. canis* have been described, there was no information on the prevalence of feline hepatozoonosis in Iran. The aim of this study was the molecular detection and identification of *Hepatozoon* in the blood of cats from six provinces.

**Methods:** From 2018 to 2022, blood samples were collected from 772 cats (299 stray, 473 client-owned) cats from both sexes and different ages in Tehran (n = 295), Mashhad (n = 239), Kermanshah (n = 85), Hamedan (n = 53), Yazd (n = 52) and Kerman (n = 48). The presence of *Hepatozoon* was detected using conventional PCR. Positive samples were further examined with Sanger sequencing and phylogenetic analysis. Statistical analysis was performed to identify risk factors associated with infection.

**Results**: 29 cats (21 stray, 8 owned) scored PCR-positive. Infected cats were found in Mashhad (n = 20; prevalence = 8.4%), Hamedan (n = 2; prevalence = 3.8%), Kermanshah (n = 2; prevalence = 2.4%) and Tehran (n = 5; prevalence = 1.7%). BLAST analysis of obtained nucleotide sequences revealed *H. felis* (n = 25) and *H. canis* (n = 3). Hepatozoonosis was significantly higher in stray cats, and in Mashhad. There was no statistically significant association between the infection and age or sex.

**Conclusion:** This research is the largest epidemiological study on feline hepatozoonosis in Iran. Further investigations on domestic and wild felids in other regions of the country, on the clinical impact of the infection, and possible presence of *H. silvestris* are suggested.

Keywords: Hepatozoon felis, H. canis, cat, vector-borne

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Hypereosinophilia, human trichostrongyliais and interference in clinical diagnosis of acute fascioliasis in Guilan province, Iran

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**Background**: Trichostrongyliasis is a zoonotic helminthic infection in herbivorous animals and humans, caused by different species of the genus *Trichostrongylus*, following ingestion of raw or undercooked vegetables carrying the infected larvae. Infections have mainly been reported among farmers throughout the world, with particularly high prevalence in Asia and the Middle East.

**Methods:** Stool samples of 500 individuals, including two family groups, who were suspected to have fascioliasis and admitted due to clinical symptoms such as abdominal pain, weight loss, urticaria and pruritus, muscle pain, diarrhea, nausea, fever and chill accompanied with hypereosinophilia were analyzed by formalin-ether, Kato-Katz and agar culture methods.

**Results**: Among the examined individuals 41 egg positive cases of fascioliasis, 8 cases of strongyloidiasis and 22 cases of trichostrongyliasis including 12 members of two families were diagnosed.

**Conclusion:** Guilan province has been included among 6 countries having serious problem with fascioliasis by World Health Organization. Hypereosinophilia is considered as important criteria of the disease in the endemic areas. Guilan province is also the main endemic region for trichostrongyliasis that causing hypereosinophilia (30-75%) and sometimes symptoms very similar

**Keywords**: Fascioliasis, trichostrongyliasis, clinical diagnosis, Guilan

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Oral

Identification and comparison of the morphological characteristic pattern of the rostellar hooks of adults and metacestode stage of *E. granulosus* and *E. multilocularis* isolated from Iran: A study based on a model proposed by the late Professor Iraj Mobdi

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**Background**: The late professor Dr. Iraj Mobdi used morphological methods to identify the genus and species of helminthic parasites, especially *Echinococcus* spp. Based on the morphological differences in *E. granulosus* from different hosts/geographical region. A number of strains have been reported.

**Methods:** The rostellar hooks of adults and metacestode stage of *E. granulosus* and *E. multilocularis* isolated from the adult worm with dogs and jackals as their definitive host and protoscloces belonging to sheep and buffalo metacestodes were collected from north, North West, south of Iran and north of India. The morphological data from each isolate was analyzed using SPSS software. The pattern of the rostral hooks of the adult and larval stage were photographed and were drawn using Camera lucida.

**Results**: Four patterns in the adult worms and three patterns of rostellar hooks were observed as follows: 1) *E. granulosus* models common in Iran, especially northwest of Iran. 2) Small *Echinococcus* comparative to *E. oligarthrus*, belonging to the carnivore's small intestine (dogs and Jackals) from Caspian Sea area. 3) Small *E. granulosus* comparative to *E. oligarthrus* and *E. vogeli*. Rostellar hooks of protoscoleces isolated from hydatid cysts. Morphological of the rostellum larval hooks were arranged to: 1) Regular (Type A) 2) Irregular (Type B) 3) Irregular (Type C).

**Conclusion:** The model proposed by the late Professor Iraj Mobedi could be used to as a morphological method to identify and diagnose the genus and species of worm parasites in this region. The results of this study could produce an attention to a question about the possible availability of the adult.

Keywords: *Echinococcus*, morphological method, rostellar hooks Correspondence Email(s): sgholami200@gmail.com



Identification of *Entamoeba histolytica* lectin antigen and the expression level of miRNA-643 and MSI in colon adenocarcinoma biopsy samples

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**Background**: *Entamoeba histolytica* is the third leading cause of death due to parasitic infections worldwide. The role of protozoa as an environmental factor in microsatellite instability (MSI) has not been well studied, and miRNAs have not been adequately studied as potential biomarkers for amoeba or in the context of colorectal disease.

**Methods:** This study involved the analysis of 150 colorectal cancer biopsy samples using techniques such as immunohistochemistry (IHC), Multiplex PCR, RT-qPCR, and Real Time-PCR to detect *E. histolytica*, miRNA-643, XIAP, and MSI. To enhance the accuracy of MSI diagnosis, both "PCR-Multiplex" and immunohistochemistry (IHC) were performed simultaneously.

**Results**: Out of the 150 biopsy samples examined, 39 cases were positive for MSI (28 cases MSI-H and 11 cases MSI-L), and 111 cases were negative for MSI. The co-occurrence of MSI and *E. histolytica* antigen was observed in 11 samples. In samples positive for both *E. histolytica* antigen and MSI, increased expression of miRNA-643 was observed, contrasting with XIAP expression.

**Conclusion:** The simultaneous occurrence of MSI and the presence of *E. histolytica* antigen, along with an increased expression of miRNA-643 compared to the XIAP inhibitor gene in colorectal adenocarcinoma biopsy samples, supports the theory of apoptosis and the role of this protozoan in the development of MSI.

**Keywords**: *Entamoeba histolytica*, miRNA-643, MSI, XIAP

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Oral

Identification of nasal and visceral *Trichobilharzia* spp. of wild migratory anatids in Guilan province in 2022-2023

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**Background**: Cercarial dermatitis is caused by various species of *Trichobilharzia*. It is considered as an emerging zoonotic disease in many parts of the world. Guilan is located along with migratory routes of waterfowls. This study aimed to determine the identification of nasal and intestinal *Trichobilharzia* spp. of wild anatids in Guilan.

**Methods:** Waterfowls required for this study were collected from local markets from different regions of Guilan province. They were then transferred to the parasitology laboratory of the Medical School of Guilan University of Medical Sciences for examination. The nasal and mesenteric veins of these birds were checked for the presence of adult worms and eggs using standard methods. Some of the isolated eggs and adult fragments were used for morphological studies and molecular characterizations. Finally, by drawing a phylogenetic tree, the obtained sequences in the present study were compared with other submitted sequences of *Trichobilharzia* species in the GenBank.

**Results**: Totally, 40 birds from various areas of Guilan province were collected. Three specimens (7.5%) were infected with nasal *Trichobilharzia* (*T. regenti*), and 28 ones (70%) were infected with visceral *Trichobilharzia*. Among the visceral *Trichobilharzia* obtained from infected birds 3 cases (10.7%) belonged to *T. querquedulae* and 25 (89.3%) to *T. franki*. The results of morphological studies confirmed the presence of polymorphism in a single species, and molecular studies revealed the concurrence of the obtained sequences with the registered genes in Iran, Europe, America, Africa, and New Zealand.

**Conclusion:** The results of the morphological study of *Trichobilarzia* spp. led to the introduction of a new species of this genus for the first time in Asia, as well as the existence of polymorphism in *Trichobilarzia* species in final host birds. Molecular analysis were completely consistent with previous studies.

Keywords: Wild anatids, PCR, ITS1, COX1 Correspondence Email(s): k\_fashi@yahoo.com



Identification of the species and genotypes of *Echinococcus* and *Taenia* in free-roaming dogs in urban areas of Kerman, southeastern Iran

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**Background**: Our knowledge on the risk of CE transmission in the urban settings in the endemic regions is limited. The present study surveyed the species and genotypes of *E. granulosus* and other taeniids by examining faeces of free-roaming dogs in the urban areas in the city of Kerman, southeastern Iran

**Methods:** The city was divided into 100 consecutive blocks of which 25 blocks were randomly selected. Fecal samples of FRDs were counted; mapped and fresh samples were collected. Then Zinc chloride flotation and sequential sieving was performed and the samples were examined under an inverted microscope. Single individual taeniid eggs were isolated, partial nad1 gene was amplified and sequenced to identify species and genotypes.

**Results**: In total 5607 fecal samples of dogs were mapped and 83 fresh samples were collected within the city. Seven out of the 25 city blocks (28%) and 10.8% of the fecal samples from FRDs were found infected with taeniid eggs. *Echinococcus granulosus* sensu lato eggs were found in four dog samples (4.8%). Two species of *E. granulosus* sensu lato were found perpetuating in the city, i.e. *E. granulosus* sensu stricto (2.4%) and *E. canadensis* (2.4%). In addition, 2 *Taenia* species including *T. hydatigena* (3.6%) and *T. serialis* (1.2%) were detected in two different blocks of the city.

**Conclusion:** This study documented the potential risk of CE transmission to humans resulting from the feces of the dogs roaming freely in urban areas. Further studies on cystic echinococcosis in dogs roaming freely in urban areas are essential to improve our understanding of the epidemiology of CE in endemic countries.

**Keywords**: Urbanization, dog, environmental contamination, cystic *Echinococcus*, taeniidae **Correspondence Email(s):** Email: fasihi@kmu.ac.ir Oral

In vivo and in vitro effects of chloroformic extracts of *Pyrus boissieriana* on *Leishmania major* 

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**Background**: Most of the drugs that are used to treat leishmaniasis have toxicity. Due to the presence of many biologically active compounds such as arbutin in Telka plant (*Pyrus biossieriana Buhse*), we decided to investigate in vitro and in vivo effects of its chloroformic extract on *Leishmania major*.

**Methods:** The chloroformic extract of the *P. biossieriana* was prepared by soxhlet method. In order to obtain the promastigotes, the standard strain of *L. major* was cultured in the RPMI1640. In the following, the effect of different concentrations (1-512  $\mu$ g/mL) of the extract on the promastigotes was investigated using flow cytometry method. In addition, after the creation of ulcers in inbred mice, concentrations of 20 and 40 mg/kg of the extract were prescribed by two methods of injection into the wound and lotion for 21 days and daily, and the area of the wounds was measured at different times.

**Results**: The results of the direct effect of different concentrations of the extract showed that the concentration of  $512\mu g/mL$  had the highest lethality (79.4%) on promastigotes of *L. major* and the concentration of 1  $\mu g/mL$  had the lowest lethality (4.01%). The calculated IC50 was 37.33  $\mu g/mL$ . We also observed significant decreases in ulcer sizes of mice treated with different concentrations of chloroformic extract ( $P \le 0.05$ ).

**Conclusion:** The results of this study showed the chloroformic extract of *P. biossieriana* has a lethal effect on the promastigotes of *L. major* in a dose-dependent manner, and reduces the size of leishmaniasis ulcers in mice. So, more researches in this field are recommended to achieve effective and safe treatment for cutaneous leishmaniasis.

Keywords: Leishmania major, Pyrus biossieriana, mice

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Vaccination with live Attenuated *L. major* and TLR4 agonist promotes IL32 cytokine and induces protection against *L. major* infection in BALB/c mice

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**Background**: *Leishmaniasis* is a skin disease which caused by different species of *Leishmania*. To date, no effective vaccine has been developed to prevent cutaneous leishmaniasis. One of the most effective vaccination methods against cutaneous leishmaniasis is leishmanization with attenuated *L. major* parasites. Toll like receptors play a major role in immune.

**Methods:** Attenuated *L. major* (MRHO/IR/75/ER) was prepared by continuous sub-culturing of the parasite. A total of 60 mice were assigned to 6 groups including of BALB/c (G1-6). Group 1 was the control groups, group 2 received the wild type *L. major* promastigotes, group 3 the attenuated line, group 4 the TLR4 agonist, group 5 the wild-type *L. major* and TLR4 agonist, and group 6 the attenuated line along with TLR4 agonist. Vaccinated mice were then challenged with wild-type of *L. major*. The expression level of IL-32 was evaluated using cytometric beadbased assay before the challenge.

**Results**: Findings of the study showed that the differences in the expression level of IL-32 in different groups of BALB/c was significant (p0.05). Vaccinated mice with a TLR4 agonist or attenuated *L. major* plus TLR4 agonist produced the highest level of IL-32.

**Conclusion:** Vaccination with the attenuated *L. major* along with TLR4 agonist promotes a Th1- mediated immune response, which leads to the protection of BALB/c mice against *L. major* infection. Therefore, it can be concluded that vaccination with attenuated strain of *L. major* and TLR4 agonist can be an efficient method to provide.

Keywords: Live attenuated *L. major*, TLR4 Correspondence Email(s): sn.noorpisheh@yahoo.com Oral

Inhibition of anti-inflammatory cytokines, IL-10 and TGF-β, in *Leishmania major* infected macrophage by miRNAs: A new therapeutic modality against leishmaniasis

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**Background**: *Leishmania major* (*L. major*) applies several mechanisms to escape the immune system. Interleukin-10 (IL-10) and Transforming Growth Factor (TGF- $\beta$ ) downregulate nitric oxide synthase (iNOS) leading to the survival of *Leishmania* within macrophages. The miRNAs are known as the modulators of the immune system. The present study was conducted to assess the effect of synthetic miR-340 mimic on cytokines (IL-10 and TGF- $\beta$ 1) involved in L. major infected macrophages.

**Methods:** The miRNAs targeting of IL-10 and TGF- $\beta$ 1 was predicted using bioinformatic tools. Relative expression of predicted miRNA, IL-10, and TGF- $\beta$ 1 was measured by RT-qPCR before and after synthetic miRNA mimic transfection. Concentration of IL-10 and TGF- $\beta$  was measured in post treatment condition using ELISA method. In addition, infectivity was assessed by Giemsa staining.

**Results**: mmu-miR-340 received the highest score for targeting cytokines. The expression of miR-340 was downregulated in *L. major* infected macrophages. By contrast, expression of IL-10 and TGF- $\beta$ 1 was upregulated in infected macrophages. After miRNA transfection, TGF- $\beta$ 1 and IL-10 were both downregulated and interestingly, the combination of miR-340 and miR-27a had a stronger effect on the downregulation of target genes.

**Conclusion:** This research revealed that transfection of infected macrophages with miR-340 alone or in combination with miR-27a mimic can reduce macrophage infectivity and might be introduced as a novel therapeutic agent for cutaneous leishmaniasis.

Keywords: Cutaneous leishmaniasis, IL-10, *Leishmania* Correspondence Email(s): a\_haghighi@sbmu.ac.ir



Investigating the therapeutic effect of *Toxoplasma gondii* on experimentally induced breast cancer in BALB/c mice

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**Background**: The immune responses against *Toxoplasma gondii* depend on IL-12 and IFN- $\gamma$ . The host cells are affected by CD4 and CD8 T cells. Reports have shown that *Toxoplasma* can suppress tumors developed in animal models. This study aims to evaluate the effect of *Toxoplasma* on the growth of murine breast tumors.

**Methods:** In this study, breast cancer was induced using the cell line MC4-L2 in BALB/c mice. The mice were then divided into control and two interventions, tachyzoite and cyst inoculation. Tachyzoite and cyst of *T. gondii* PRU strain were injected into the tumors. The measures of the tumors were evaluated with a vernier caliper and ultrasound. Subsequently, tumor cells and tissues of the liver, lymph nodes, sternum, and brain were histologically examined. Moreover, *Toxoplasma* infection was assessed by IgG antibody using the MAT method.

**Results**: There were no significant differences in tumor size between the two intervention groups and the control group with the vernier caliper (P = 0.005). However, ultrasound imaging showed a significant relationship between the intervention group with cysts and the control group (P = 0.001). In the histopathology of the samples, no metastases were seen in the control and intervention groups. *Toxoplasma* cysts were observed only in the brain tissue of a mouse from the tachyzoite intervention group.

**Conclusion:** Statistical analysis showed that injection of *Toxoplasma* cysts into the tumor has an inhibitory effect compared to the control group. We suggest that more studies will be undertaken on related immunological factors in the future.

Keywords: Toxoplasma gondii, breast cancer, tumor Correspondence Email(s): asgarig@sums.ac.ir Oral

#### Investigation of persistence of infectious *Sarcocystis* spp. and *Toxoplasma gondii* in raw meat product of markets using Real-time PCR

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**Background**: *Toxoplasma gondii* and *Sarcocystis* spp as an obligatory intercellular parasite in mammals. Eating of undercooked or meat products can cause infection in human. This study aimed to investigate the *Sarcocystis* spp. and *T. gondii* infection rate in processed meat products in Tehran.

**Methods:** A total of 160 samples, including hamburgers, sausages, and Bologna were collected from 5 plant (A ... E) Products marketed in Tehran, Iran and examined by peptic digestion and microscopy method and molecular method (Real-time PCR). All samples were stained by Giemsa staining method and observed microscopically for detecting *Sarcocystis* and *T. gondii* bradyzoite.

**Results**: In the present study, only *Sarcocystis* was detected in different types of meat products. The results shown that 45/160 (28.1%) of all samples tested were infected with *Sarcocystis* spp. From 45 positive samples, hamburgers (10/45), sausages (15/45), and Bologna (20/45). The presence of *T. gondii* DNA in meat products were not seen using Molecular assays.

**Conclusion:** Therefore, increased knowledge of *Sarcocystis* and *Toxoplasma* prevalence in the meat products can be helpful to predict the infection rate in humans and to show the necessity of improving parasite transmission prevention.

**Keywords**: *Toxoplasma gondii*, *Sarcocystis* spp, meat products

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Knocking down the expression of the molecular motors, myosin A, C and F genes in *Toxoplasma gondii* to decrease the parasite virulence

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**Background**: Toxoplasmosis is a serious parasitic infection and novel therapeutic options are highly demanded to effectively eliminate it. In current study, *Toxoplasma gondii* myosin A, C and F genes were knocked down using small interference RNA (siRNA) method and the parasite survival and virulence was evaluated in vitro and in vivo.

**Methods:** The parasites were transfected with specific siRNA, virtually designed for myosin mRNAs, and cocultured with human foreskin fibroblasts. The transfection rate and the viability of the transfected parasites were measured using flow cytometry and methyl thiazole tetrazolium (MTT) assays, respectively. Finally, the survival of BALB/c mice infected with siRNAs-transfected *T. gondii* was assessed.

**Results**: It was demonstrated that a transfection rate of 75.4% existed for siRNAs, resulting in 70% (P = 0.032), 80.6% (P = 0.017) and 85.5% (P = 0.013) gene suppression for myosin A, C and F in affected parasites, respectively, which was subsequently confirmed by Western blot analysis. Moreover, lower parasite viability was observed in those with knocked down myosin C with 80% (P = 0.0001), followed by 86.15% (P = 0.004) for myosin F and 92.3% (P = 0.083) for myosin A.

**Conclusion:** Considerably higher mouse survival (about 40 h) was demonstrated in mice challenged with myosin siRNA-transfected *T. gondii*, in comparison with control group challenged with wild-type parasites. In conclusion, myosin proteins knock down proposes a promising therapeutic strategy to combat toxoplasmosis.

Keywords: Expression, myosin, virulence, siRNA, Toxoplasma

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Oral

Lactobacillus & Bifidobacterium of patients with strongyloidiasis compared to the control group

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**Background**: In individuals with compromised immune systems, strongyloidiasis disease can lead to disseminated infections that can be fatal if diagnosis and treatment are delayed. The human gut is composed of numerous bacteria, which play essential roles in nutrient absorption, synthesis of necessary substances, absorption of organic compounds, development of acquired immunity.

**Methods:** This case-control study was conducted in the year 2023. Stool samples were collected from individuals who referred to the Diagnostic Laboratory of Strongyloidiasis in the School of Public Health, Tehran University of Medical Sciences, compared with the control group. After DNA extraction, the 16SrRNA gene was examined using Real- time PCR. The levels of *Lactobacillus acidophilus* and *Bifidobacterium bifidium* were calculated in both groups. Finally, statistical analysis was performed.

**Results**: Out of 28 participants in this study, 16 (57%) were men and 12 (43%) were women, with age ranging from 43 to 76 years. A statistically significant relationship was observed between underlying diseases, vegetable washing practices, and clinical symptoms of strongyloidiasis in this study. The average number of L. acidophilus and *B. bifidium* were (1012) (132533/3  $\pm$  07250/4) and, (1012) (519169/3  $\pm$  12857/6) in the case group respectively, which were lower compared to the control group, that had 1012× (542372/6  $\pm$  04733/7) and 1012× (754185/4  $\pm$  36643/8) respectively. The odds ratio was for *L. acidophilus* and *B. bifidium* 1.13 and 1.14,

**Conclusion:** It was observed that for each increase in the number of 1012 in the microliter from for *L. acidophilus* and *B. bifidium* in the individual's intestines in areas endemic to strongyloidiasis, the chances of contracting this disease decreased by 13% and 14%, respectively. Future studies with a higher volume considering age,

**Keywords**: *Bifidobacterium bifidium*, *Lactobacillus acidophilus* **Correspondence Email(s)**: zfk579@gmail.com



Low occurrence of pulmonary lophomoniasis among cigarette smokers: A registry -based evidence

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**Background**: *Lophomonas* is an emerging protozoan agent that mainly causes pulmonary lophomoniasis. Cigarette smoking also causes a vast majority of complications. Yet there are few diseases, which have lower prevalence in smoker population. Here we conducted a study to investigate prevalence of *Lophomonas* infection among smokers in competition with non-smokers.

**Methods:** This preliminary retrospective study conducted through 2023, which analyzed 135 *Lophomonas* positive patient smoking status. All patients were registered in the Iranian National Registry Center for Lophomoniasis (INRCL).

**Results**: We studied 135 *Lophomonas* positive patients (male = 92, female = 43). We found a statistically significant occurrence of lophomoniasis among non-smokers (70.4%; n = 95), in contrast to smokers (29.6%; n = 40) (P = 0.001).

**Conclusion:** Our findings reveal that *Lophomonas* infection is significantly lower among cigarette smokers compared to non-smokers. Our data shed light on a new hypothesis about lophomoniasis among smokers. Further research is needed to assess the role of smoking in the pathophysiology of lophomoniasis.

**Keywords**: cigarette smokers, lophomoniasis, registrybased study

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Oral

Metabolic changes of *P. berghei* in the presence of novel nanocomposite (NDC-CQ)

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Tarbiat Modares University, Tehran, Iran Background:. The present study aimed to examine antimalarial effects

of a novel synthesized nonochloroquine-loaded curcumin relying on dendrimer G2.

**Methods:** Subsequent to synthesis of nanocomposite, characterization was carried out NMR, LC-MS, scanning electron microscopy, dynamic light scattering and Fourier-transform infrared. The mice were infected by *P*. berghei ANKA ( $2 \times 106$  parasitized erythrocytes) intraperitoneally. After observing parasitemia in the blood, the drugs were administered intraperitoneal route. After 24h, blood samples were collected from anesthetized mice and serum isolated by centrifugation, and samples transferred to the Central Laboratory of University of Isfahan, Iran. Knight and Peters' proposed method was used as the treatment basis.

Results: Subsequent to synthesis of nanocomposite, characterization was carried out NMR, LC-MS, scanning electron microscopy, dynamic light scattering and Fourier-transform infrared. 25 male BALB/c mice were selected and divided into 5 groups (5 in each). They were included as follows: a negative control administered with normal saline, a positive control was injected with 200 mg/kg CQ, and the rest of the sample groups were injected with 10, 20, 50 mg/kg NDC-CQ respectively for four days. At the first, the mice were infected by P. berghei ANKA (2×106 parasitized erythrocytes) intraperitoneally. After observing parasitemia in the blood, the drugs were administered intraperitoneal route. All cases were injected once daily in 24 h intervals. The blood samples were taken at the end of the fourth day via tail vein sampling. The blood samples were collected from anesthetized mice and serum isolated by centrifugation, and samples transferred to the Central Laboratory of University of Isfahan, Iran. Knight & Peters' proposed method was used as the treatment basis.

**Conclusion:** Due to the better effect of the synthesized nanocomposite on *P. berghei* in mice compared to chloroquine in this study and previous research, this nanocomposite can be considered as an effective anti-*Plasmodium* compound while more comprehensive research is recommended.

Keywords: Nanocomposite, *P*. berghei, chloroquine, mice Correspondence Email(s): tabatabaie59@gmail.com tabatabaei.f@iums.ac.ir



Microscopic and Molecular Identification of *Echinococcus multilocularis* and *Echinococcus granulosus* among carnivorous in Guilan province, Iran

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**Background**: Alveolar echinococcosis, a lethal zoonotic disease, is caused by *Echinococcus* multilocularis, a small tapeworm from wild/domestic carnivores. This study assess E. *multilocularis* and *E. granulosus* in carnivorous animals from Guilan province, Iran that is of particular concern due to high influx of tourists, increasing the risk of transmission to humans.

**Methods:** A total of 272 fecal samples from carnivores were collected across 36 different regions in Guilan province, with the majority collected from late winter (March 2022 to June 2023). The samples underwent direct wet mount microscopic examination and concentration techniques (formal-ether) followed by polymerase chain reaction (PCR) analysis. To detect the presence of *E. multilocularis* and *E. granulosus* eggs, PCR primers were designed to amplify genes of mitochondrial cytochrome c oxidase subunit 1 (cox1) and NADH dehydrogenase subunit 1 (nad1). Out of all the samples examined, 24 (8.82%) tested positive for E. *multilocularis* and/or *E. granulosus*.

**Results**: From examined samples, 24 (8.82%) positive for E. *multilocularis* and/or *E. granulosus*. Small intestines of five jackals were examined, revealed one *Mesocestoides* spp. through intestinal scraping technique (IST) and the sedimentation counting technique (SCT). A total of 271 samples successfully amplified using PCR, with E. *multilocularis* and *E. granulosus* infections detected in four (1.47%) and 68 (25.0%) fecal samples from Guilan province, respectively. Furthermore, four jackals were only infected with E. *multilocularis* (5.06%), while two jackals displayed co-infections of both *E. multilocularis* and *E. granulosus* (2.53%). *E. granulosus* was found in the fecal samples of dogs (23.43%) and jackals (29.11%).

**Conclusion:** Comparing microscopic and formal-ether with PCR, displayed higher frequency/sensitivity (26.56%) of PCR than traditional techniques (8.82%). Life cycle of E. *multilocularis* and E. *granulosus* among carnivores in Astara, Talesh, and Bandar Anzali documented them as final hosts and sequencing analysis confirmed *Echinococcus granulosus* (G1) was dominant genotype in this region.

Keywords: *E. multilocularis, E. granulosus*, PCR Correspondence Email(s): hfeizhaddad@yahoo.com Oral

Molecular and morphological study of *Clinostomum complanatum* (Digenea: Clinostomidae) in Aras River from Iran

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**Background**: This study was performed to morphological and molecular differentiation of yellow grub infecting edible fishes in Aras River from Iran.

**Methods:** In this study, 100 fish belonging to the nine species were collected from Aras River in 2021-2022. The samples were analyzed using the combination of morphological and molecular techniques for infection with *Clinostomum complanatum* metacercariae. The isolated metacercariae from recovered cyst were fixed with 70% ethanol and clarified with amman's lactophenol and staining by azocarmine. The total DNA of isolate metacercariae was extract using a Qiagen extraction kit, according to the manufacturer's instructions. The PCR was performed by the partial mitochondrial COX-I gene with 620 bp size was amplified.

**Results**: Results: The metacercaria were identified only from the specimens belonging to *Cyprinus carpio* with the prevalence of 5%. The morphologic features of isolated *C. complanatum* metacercariae were as follows: narrowed around the ventral sucker, the small Oral sucker, well developed Oral collar, the extended intestinal ceca until ventral sucker, triangular testes, the well-developed cirrus sac that opens into the genital pore and irregular ovary. The sequencing of amplified fragments confirmed morphological study as all metacercariae was belonging to *C. complanatum* with homology of 100% with other sequences of *C. complanatum* available in the NCBI database (accession No: OP984764).

**Conclusion:** The larva of *C. complanatum* can cause pharyngitis or laryngitis in human if they were consumed. However, in different part of the world especially in Asian countries where it is common to eat raw fish such as sushi, people may become infected with this parasite.

Keywords: *Clinostomum complanatum*, *Cyprinus carpio* Correspondence Email(s): zahra61.h@gmail.com za.heidari@arums.ac.ir



Molecular characterization of *Acanthamoeba* isolated from soil samples and malignant patients from Zanjan province, northwest of Iran

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**Background**: The genus *Acanthamoeba* is reported from various environmental sources. It can cause multiple complications, including Chronic Amoebic Keratitis (CAK) and Amoebic Granulomatous Encephalitis (GAE). This study investigated the presence and genotyping of *Acanthamoeba* in the soil of parks and patients with malignancies referred to health centers in Zanjan province, Iran.

**Methods:** In this cross-sectional study, 200 soil samples were collected from amusement parks in Zanjan city from September 2017 to May 2018. Samples were cultured on 1.5% Non-Nutrient Agar, and the *Acanthamoeba* genus was identified using the morphological method. PCR was performed on all positive environmental samples, and six microscopically positive clinical samples belonged to our previous study. DNA sequencing of 18S rRNA was performed to analyze the genetic pattern of some PCR-positive isolates.

**Results**: Microscopic results showed that 96 (48.00%) soil samples were positive. PCR confirmed all positive cases of clinical samples and 84 soil samples. Out of the PCR-positive samples, 20 soil samples and 5 clinical samples were sequenced successfully. All soil isolates belonged to the T4 genotype, and three and two clinical samples belonged to T4 and T5 genotypes, respectively.

**Conclusion:** The presence of *Acanthamoeba* in both the environment and clinical samples of Zanjan city suggests paying greater attention to the infections caused by it.

Keywords: *Acanthamoeba*, malignancy, soil, Zanjan, Iran Correspondence Email(s): apezeshki@zums.ac.ir

Oral

Molecular diagnosis of *Leishmania* parasites isolated from cutaneous leishmaniasis patients in Esfarayen county, north Khorasan province, northeastern Iran, 2023

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**Background**: Cutaneous leishmaniasis (CL) is an endemic disease in the Middle East, including Iran, and the disease is spreading to new foci. We aimed to describe leishmaniasis causing CL in Esfarayen county.

**Methods:** Overall, 122 suspected CL patients admitted to medical centers in Esfarayen County North Khorasan Province, Northeastern Iran. In Late 2023 And Early 2024, we consecrated all patients and tested them for *Leishmania* infection through clinical and molecular testing. Seventy three patients had a positive smear for CL based on optical microscopy. Total DNA was extracted using proteinase K and the phenol/chloroform/isoamyl alcohol method. Different products of kinetplastid DNA minicircles were amplified by nested PCR using species-specific primers (LIN R4-LIN 17-Lin 19).

**Results**: A total of 73 smears (%59) were positive for Leishman bodies under the optical microscope. The Parasite was determined to be *Leishmania major* (*L. major*) by nested PCR. Skin lesions and scars caused by CL mostly appeared on the patient's hands and face. Additionally, more than two skin lesions were observed in 50 cases (68%), all of which were infected with *L. major*. A single skin ulcer was seen in 23 (32%) of the CL patients.

**Conclusion:** Zoonotic disease or rural cutaneous leishmaniasis is common in Esfarayen county whose pathogen species is *L. major*. Molecular analysis using specific primers is accurate method for detecting *Leishmania* species.

Keywords: *Leishmania major*, Nested PCR Correspondence Email(s): alp.shaghayegh1995@gmail.com aliehsan2001@yahoo.com



Molecular epidemiology and associated risk factors of *Trichomonas tenax* and *Entamoeba gingivalis* in pregnant women in western Iran

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**Background**: We aimed to study the frequency and risk factors of Oral cavity protozoa (*Trichomonas tenax* and *Entamoeba gingivalis*) in pregnant women in Lorestan province, western Iran.

**Methods:** The current cross-sectional descriptive work was performed on 200 pregnant women referring to health centers of Lorestan Province, Iran during August 2021 to February 2022. Two specimens were obtained from each woman by means of sterile swabs from saliva and dental plaques. The frequency of Oral cavity protozoa was investigated using microscopic and conventional polymerase chain reaction (PCR).

**Results:** *E. gingivalis* and *T. tenax* parasites were found in 41 (20.5%) and 46 (23%) of the pregnant women by microscopic and PCR test, respectively. Among positive samples, 29 (63.1%) of the pregnant women were infected with *E. gingivalis*; whereas 19 (36.9%) of the participants were positive for *T. tenax*. In the multivariate model, living in rural regions (*P* 0.001) and brushing teeth (P = 0.021) were considerably linked with the prevalence of Oral cavity parasites.

**Conclusion:** The current study evidently exhibited the high frequency of Oral cavity parasites (*E. gingivalis* and *T. tenax*) in pregnant women in Lorestan province, Western Iran. Awareness of the main risk factors for Oral cavity parasites especially teeth brushing is necessary in refining public and Oral health approaches in pregnant women.

**Keywords**: Prevalence, oral cavity, PCR, Iran **Correspondence Email(s):** dr.kamran3519@gmail.com Oral

Molecular identification and genotyping of *Fasciola* cercariae in field-collected Lymnaeid snails in northwestern provinces of Iran

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**Background**: Snails of the Lymnaeidae family are the intermediate hosts of *Fasciola* species; the causative agents of fascioliasis. The purpose of this study was to investigate the phylogenetic relationship of the larval stages of Fasciola isolated from Lymnaeid snails with isolates from other regions of the world.

**Methods:** A total of 2000 Lymnaeid snails were collected from 33 permanent and seasonal habitats in northwestern Iran during June to November 2021. After identification by standard morphological keys, they were subjected for shedding and crushing methods. Different stages of *Fasciola* obtained from these snails were determined by ITS1 PCR-RFLP method. A fragment of the Cox1 gene of *Fasciola* isolates was amplified and sequenced in both directions. The sequenced fragments were aligned; the phylogenetic tree of these isolates and the corresponding isolates from GenBank was generated using MEGA6 software by maximum likelihood method.

**Results**: Thirty-eight (1.9%) out of 2000 Lymnaeid snails examined were infected with cercariae of trematodes. Twenty-five of the collected snails (1.25%) were infected with *Fasciola* cercariae; Fasciola hepatica (76%) and *F. gigantica* (24%). The sequence of the cox1 fragment of *F. hepatica*: isolates was completely identical and similar to *F. hepatica*: specimens isolated from different hosts in Denmark, Austria and Australia. While the sequence of this fragment in *F. gigantica* cercariae was classified into, 5 new sequence types. The sequence of the Cox1 gene of *F. gigantica* cercariae was most similar to adult worms isolated from cattle in Turkey.

**Conclusion:** In this study, the isolates of F. *gigantica* cercariae had significant intraspecies variation in the cox1 sequences, while no variation was observed in the sequence of this gene in F. *hepatica* cercariae.

Keywords: Fasciola, lymnaeidae, genotypes, northwestern Iran

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Molecular identification of animal *Fasciola* and *Dicrocoelium* isolates from two center regions of Iran

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**Background**: Fascioliasis and dicrocoeliasis are two important zoonotic diseases in humans and ruminants. The purpose of this study was to detect the genetic diversity of Fasciola and Dicrocoelium spp. using the PCR-RFLP method. In addition, genetic diversity and phylogenetic analysis were evaluated using 18S, 28S, ITS (rDNA) ND1 and CO1 (mtDNA) markers.

**Methods:** A total of 160 and 200 Iranian *Fasciola* and *Dicrocoelium* isolates were collected from infected cattle, sheep, and goats at two abattoirs in the center of Iran to identify them using PCR-RFLP and sequence analysis of the 18S, 28S, ITS (rDNA) ND1 and CO1 (mtDNA) loci's. PCR products of ITS2, 28s of *Dicrocoelium* samples, as well as 28s, CO1, and ND1 of *Fasciola* samples, were subjected to digestion by Bfa1, TruiI, BsrB1, ECO881, and Hind III fast restriction enzymes, and visualized on agarose gels. DNA from 30 isolates of *Fasciola* and 30 *Dicrocoelium* of different hosts were sequenced and evaluated.

**Results:** The PCR reaction showed the length of 18S, 28S, ND1, CO1 of *Fasciola* at 260bp, 618bp, 700bp, and 500bp, and the length of the ITs2 and 28S of *Dicrocoelium* was 236bp and 963bp respectively. *D. dendriticum* has a RFLP pattern of 110, and 126bpb and size (ITS2), as well as 116, 293, 409bp (28s) using, Bfa1 and Tru1I restriction enzymes. *F. gigantica* has a profile of 333, and 285bp (28s) using Bsrb1 restriction enzyme. The RFLP pattern of genotype *F. hepatica* was 73, 120, and 507bp (ND1) and 119 and 381bp (CO1) in size using Hind III and ECO881 restriction enzymes.

**Conclusion:** Using the PCR-RFLP method, two species of *F. hepatica* and *F. gigantica*, and only *D. dendriticum* were present in the center of Iran and caused infection in cattle, sheep, and in goats. Data from this study provides information that serves as the basis for further studies in other regions.

Keywords: PCR-RFLP, Fasciola, Dicrocoelium

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Oral

Morphological and molecular identification of *Toxocara* isolated from road-killed golden jackals in Northern Iran

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**Background**: Toxocariasis is caused by infection with *Toxocara canis* and *Toxocara cati*, common nematodes of canids and felids, respectively. Humans become infected after the accidental ingestion of embryonated eggs of *Toxocara* from the soil or the consumption of raw and undercooked meat containing *Toxocara* larvae.

**Methods:** This cross-sectional study was conducted on 41 roadkilled golden jackals collected from Guilan and Mazandaran provinces in northern Iran. At first, species identification was carried out based on morphological characterization. Genomic DNA was extracted from the isolates of *Toxocara* collected from jackals. PCR-RFLP of Ribosomal DNA regions (ITS) using RsaI endonuclease enzyme and PCR-sequencing were carried out to identify *T. canis*. The sequence data were aligned using Bioedit software and compared with published sequences in GenBank using the BLAST system. Phylogenetic analysis was performed using MEGA 5.0 software.

**Results**: Eleven out of 41 road-killed golden jackals (26.8%) were infected with *Toxocara* nematodes. All the isolates were confirmed as *T. canis* based on morphological and molecular methods. A pairwise comparison of the sequences did not show any differences in nucleotide sequences within *T. canis* isolates, and the sequences were identical and exhibited 100% homology.

**Conclusion:** Considering the almost high prevalence of *T. canis* and its critical role in human toxocariasis, the identification of parasite species by molecular methods can be used to plan prevention and control programs in human and animal communities. The type of host and geographical region do not affect the genetic diversity.

Keywords: Morphology, molecular identification, *Toxocara*, jackal

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Oral

Morphological and molecular investigation of parasitic nematodes of the Anisakidae family in Persian Gulf fishes, Hormozgan province

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**Background**: Over recent decades, substantial advancements in the food industry have occurred, leading to increased exposure to unidentified allergens. Concurrently, societal shifts in eating habits, particularly the prevalent consumption of raw, salted.

**Methods:** The aim of this investigation was to explore parasitic nematodes belonging to the Anisakidae family in fish from the Persian Gulf within Hormozgan province. A total of 460 fish specimens representing 14 distinct species underwent examination for disease and parasitic lesions. These species encompassed 70 flounder, 30 Otolithes ruber, 50 Lutjanus johnii, 35 Rachycentron canadum, 35 Scomberomorus commerson, 40 Pomadasys kaakan, 30 Thunnus tonggol, 35 Epinephelus, 20 Scomberoides, 40 Lethrinus nebulosus, 15 Eleutheronema tetradactylum, 25 Rastrelliger kanagurta, 10 Sparidentex hasta, and 15 Saurida tumbil. The assessment involved both morphological and molecular examinations to determine contamination with the Anisakidae family.

**Results**: Among the 460 scrutinized fish samples, 64% exhibited infection with at least one species belonging to the Anisakidae family. The examination revealed the isolation and identification of five distinct nematode species from the Anisakidae family, recovered from various anatomical locations within the fish, including the intestine, peritoneal cavity, liver, and mesentery. These identified species encompassed Anisakis simplex larvae (18.68%), *Pseudoterranova* sp. (13.68%), *Contracaecum* sp. (12.14%), *Raphidascaris acus* (11.27%), and *Hysterothylacium* (8.23%).

**Conclusion:** The Anisakidae family, parasitic nematodes in marine organisms, can cause anisakiasis a zoonotic disease transmitted to humans through raw or undercooked seafood consumption. Given the potential health risks associated with this parasite, including the development of asthma and food allergies in hosts, there is a pressing need for heightened awareness.

**Keywords**: Anisakidae, fish, Persian Gulf, parasitic **Correspondence Email(s):** zahra.gharibi65@yahoo.com Oral

May 21 - 23, 2024

Morphological isolation of free living amoebae (Acanthamoeba, Vahlkampfiids and Vermamoeba) from swimming pools in Karaj city

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**Background**: Free-living amoebae (FLA) are eukaryotic microorganisms with worldwide distribution. Because of opportunistic feature, it is important to investigate the frequency of FLA from high-traffic places. This study aimed to investigate the frequency of FLA in swimming pools as a potential place where people interact more.

**Methods:** During August to October 2023, 45 samples were collected from different parts of 15 indoor water recreation in Karaj city, Iran. These samples include sauna biofilm (n = 15), jacuzzi (n = 15) and pool water (n = 15). After filtration of water samples with 0.45-micron filters, the membranes were transferred in 1.5% non-nutrient agar medium and incubated at room temperature. In addition, to isolate thermo tolerant amoebae additional NNA medium culture was incubated in 42-45 °C for a week. The morphological detection was done under a light microscope using page key.

**Results**: Out of 45 samples that were collected from different indoor water recreation places in Karaj city, 27 samples (60%) were positive for FLA, including 9 (33.3%) of sauna biofilm, 8 (29.6%) of jacuzzi water and 10 (37%) of pool water. *Acanthamoeba* spp. was characterized in 13 (48%) samples. Twelve samples (44.4%) were positive for Vahlkampfiidae. *Vermamoeba vermiformis* was identified in ten samples (37 %), and two samples (7.4%) were positive for *Thecamoeba*. Several plates showed mixed amoebae growth.

**Conclusion:** Our findings suggest that FLA can exist in indoor swimming pool waters and high temperature jacuzzi. This issue indicates the importance of improving the sanitation and decontamination of water sources specially places where people are in close contact with them.

Keywords: Free-living amoebae (FLA), swimming pool

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Morphometric, molecular and phylogenetic characterization of *Linguatula* nymphs from domestic ruminants in Mazandaran province

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 Background: Linguatula serrata (L. serrata), also known

as tongue worm, is a Pentastomida that has a worldwide distribution. The objective of the present study was to investigate the morphometric, molecular characterization and phylogenetic analysis of nymphal stage of *Linguatula* from domestic ruminants (cattle, goats and sheep) in Mazandaran province.

**Methods:** The mesenteric lymph nodes were collected from various ruminants at Mazandaran slaughterhouses. The morphometric characteristics of six variables (hook, opening of hook, handle of hook, distance between two spines in fifth segment, the length of the spine 1 and the length of the spine 2) of the nymphs were measured in three animals (cattle, sheep and goat) using the camera Lucida and scanning electron microscope (SEM). To molecular and phylogenetic characterization, the 18 S rRNA gene was used using specific primers.

**Results**: The morphological characteristics of *Linguatula* spp. displayed variations in size among different ruminants. Through SEM examination, it was observed that the head of *Linguatula* is cone-shaped with an obtuse apex pointing ventrally in all specimens. Molecular evidence further confirmed that all nymphs identified belonged to *L. serrata*, with a 95-100% similarity compared to other *L. serrata* species.

**Conclusion:** Our study suggests that 18S rRNA gene sequencing can be a suitable method for analyzing the phylogenetic relationships of *L. serrata* among different hosts in various parts of Iran. This approach could potentially be beneficial for infection control and prevention strategies.

Keywords: 18S rRNA, Iran, Linguatula serrata

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Oral

Neutrophil extracellular traps formation: effect of *Leishmania* major promastigotes and salivary gland homogenates of *Phlebotomus papatasi* in human neutrophil culture

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**Background**: In leishmaniasis, a series of immune responses occurs after an infected bite of phlebotomine sand flies, among which neutrophils can be mentioned as the initiators with multiple functions. Here, neutrophil extracellular traps were studied in the presence of *L. major* and SGH of Ph. papatasi alone and together.

**Methods:** The effect of *L. major* and SGH on NETs formation was studied in three different groups: neutrophils + SGH (NS), neutrophils + *L. major* (NL), neutrophils + *L. major* + SGH (NLS) along with negative and positive controls in 2, 4 and 6 hours post-incubation. Different microscopic methods were used to visualize NETs comprising: fluorescence microscopy by Acridine Orange/ Ethidium Bromide staining, optical microscopy by Giemsa staining and scanning electron microscopy.

**Results**: All three microscopical methods revealed similar results, as in NS group, chromatin extrusion as a sign of NETosis, was not very evident in each three time points; but, in NL and especially NLS group, more NETosis was observed and the interaction between neutrophils and promastigotes in NL and also with saliva in NLS group, gradually increased over times.

**Conclusion:** Hence, it was determined that the simultaneous presence of parasite and saliva in NLS group has a greater impact on the formation of NETs compared to NL and NS groups.

Keywords: NETs, L. major, Ph. papatasi Correspondence Email(s): yasamaryan@gmail.com



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Oral

Phylo-molecular analysis of *E. vermicularis* in appendectomy specimens from Iran

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**Background**: Human infection with *Enterobius vermicularis* occurs worldwide, particularly in children. The role of *E. vermicularis* in appendicitis is neglected. This study was designed to investigate genotypes of *E. vermicularis* detected from appendectomy specimens in the human population from Iran and clarify the intra-species variation of the parasite.

**Methods:** Seventy appendectomies for acute clinical appendicitis isolates from Azerbaijan and North Khorasan of Iran were used in the present study. The genetic information of Tehran and Hamedan regions was also obtained from GenBank for comparison and analysis. The nucleotide sequence of cytochrome c oxidase subunit 1 (Cox1) gene was analyzed to perform genetic differentiation, haplotype network analysis, and population structure.

**Results**: Phylogenetic analysis of all the isolates were included in type B haplogroup. The number of haplotypes in all geographical locations of Iran is not much. Network analysis of sequences for regions such as Thailand, Iran, Denmark, and Poland show three classified subtypes B1, B2, and B3 in the B haplogroup.

**Conclusion:** haplogroup. It seems that the haplotypes of *E. vermicularis* detected from appendectomy are B type, and divided into three subtypes. Further research using another genetic marker is required to elucidate the genetic variation of the parasites in detail.

**Keywords**: *Enterobius vermicularis*, appendectomy, Cox1, phylogenetic

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Oral

Prediction of anticancer properties of *Toxocara canis* derived peptides using in computer based tools; an in silico and in vitro study

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**Background**: Due to the potential anti-cancer effect of helminthic parasites, in the present study, the excretory-secretory protein of *Toxocara canis* (*T. canis*) parasite was used to evaluate the possible anti-cancer properties in the in silico and in vitro (cell culture) condition.

**Methods:** The amino acid profile of the excretory proteins of the parasite was determined and the prediction of the anticancer potential of the amino acid sequences of the peptides was checked in the online database (<u>http://crdd.osdd.net/raghava/cancerppd/</u>). After checking the allergenicity and physicochemical properties of peptides, the sequence of 17 amino acids was selected. The synthesized selected peptide was exposed to cancer cell lines and the toxicity/cell viability and involved genes in tumor progression or suppression were evaluated by colorimetric and molecular techniques.

**Results**: RNRQEEEQRRQRREADRL amino acid sequence was selected from *Toxocara* Troponin T protein due to high degree of similarity ( $\approx$  93%) to other anti-cancer agents. The intended peptide was not estimated to be allergenic and the synthesized peptide with 97% purity showed anticancer properties after 24 and 48 hours in different concentrations (32, 64, 128 and 256µg/ml) when exposed to cancer (AGS, HT-29, and Caco2) and normal (HDF) cell lines.

**Conclusion:** Computer-based tools were cost-effective and timeefficient with high predictive accuracy and similar to in vitro results. Although the studied peptide at high concentrations could have a statistically significant effect on cancer cells, it is still far from the standard drug and can be optimized and promising in future studies.

**Keywords**: Anti-cancer, *Toxocara canis*, peptide, gastrointestinal, Real-time PCR



Prevalence and associated risk factors for *Giardia lamblia* infection among patients suffering from diarrhea referred to Wardak province, Afghanistan

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**Background**: *G. intestinalis* is causative of gastrointestinal infections worldwide. Contaminated food, feces, drinking water (orofecal route) and factors like economic condition, culture and behavioral factors have been involved in their transmission. Wardak province located in the central region of Afghanistan.

**Methods:** A total of 274 patients with diarrhea referred to Wardak hospital and out of them 17 individual (10(47.6%) male and 7(63.6%) female) detected positive for Giardia spp. by microscopy during 2023. In this cross-sectional study, socioeconomic, cultural and symptomatology information were collected. The association between the risk factors and intestinal parasitic infections was analyzed by Chi-Square and fishes exact tests using the SPSS 26 software and Graph pad prism 8 at a significance level of  $P \le 0.05$ .

**Results**: More than half of the patients were under 30 years old. Most cases of the disease were seen in hot seasons (spring and summer). No significant correlation was observed between the prevalence of giardiasis with having an animal at home, use of well water, economic status, and travel history. 82, 41, 52, 94 and 76% of the patients had fever, nausea, vomiting, abdominal pain, headache, respectively. 85% of patients had watery diarrhea and 15% of patients had bloody diarrhea.

**Conclusion:** This was the first epidemiological study conducted in Wardak province, center of Afghanistan. The findings revealed a pressure of giardiasis, and its interactions with multiple risk factors were investigated. This study suggested that giardiasis is important causative factor of gastrointestinal diseases in the study region.

Keywords: Giardiasis, Afghanistan, risk factor, Wardak

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Oral

Prevalence and clinical aspects of *Trichomonas vaginalis* infection among high-risk women in Karaj, Iran

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**Background**: Trichomoniasis is a common sexually transmitted infection (STI) caused by the protozoan *Trichomonas vaginalis* (*T. vaginalis*), which causes health and emotional damages to the sufferers annually. Present research, was aimed to have investigated the prevalence of *T. vaginalis* and its related risk factors among the high-risk women in the city of Karaj.

**Methods:** This cross-sectional study was conducted between October 2021 and September 2022, in total 192 samples were taken from high-risk women who were referred to the center for vulnerable women and from women in Fardis Prison of Karaj. All samples were examined by microscopic method and the results were analyzed by SPSS statistical analysis software (V.21).

**Results**: The overall prevalence of *T. vaginalis* in high-risk women was estimated at 7.8% (15/192). Subgroup prevalence was also assessed according to the severity of symptoms, and no significant association was observed between the prevalence and the symptoms severity.

**Conclusion:** Due to the high prevalence of the parasite among the vulnerable/high risk women, particularly in people with poor socioeconomic condition, preventive health measures in this high-risk group seem necessary. Nevertheless, given that men have no symptoms but may be carriers of the parasite, the same study is also recommended for

Keywords: Trichomonas vaginalis, epidemiology, high-risk women, Iran

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Prevalence of cercarial dermatitis in rice farmers of Mazandaran province

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**Background**: Cercarial dermatitis or swimmer's itch is a type of skin lesion caused by of birds or non-human mammals schistosomes especially *Trichobilarzia* genus in humans. The cercariae of many of these species can cause swimmer's itch when they penetrate human skin.

**Methods:** This descriptive cross-sectional study was conducted in the habitat of aquatic migratory birds in the villages of Sari, Faridunknar, Babolsar and Qaemshahr counties, Mazandaran, Iran. To detect human cases of cercarial dermatitis, from May 2021 to August 2021, the feet and hands of all rice farmers (n = 951) were examined for the occurrence maculopapular rashes or eruptions by interview and observation. Clinical signs and symptoms were recorded.

**Results**: Of the 951 examined people, 588 (61.8%) had macular or papular rashes on their feet and/or hands (mainly (63%) on the feet). People reported burning and itchy feeling after water drops had dried on their skin. Symptoms started with erythematic features, itching, and several hours later, maculopapular rashes became visible. The majority of people (63.5%) were adult females. Most cases occur from July to August due to shedding cercariae and peak farmer activity on rice farms and people bathing in man-made ponds.

**Conclusion:** The high prevalence of cercarial dermatitis in the rice farmers of these areas has turned this disease into a health problem. It suggests the reappearance of the disease in the areas under investigation. Therefore, it is possible to improve the health status of the region by increasing the

Keywords: Trichobilharzia, cercaria dermatitis

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Oral

Prevalence of *Sarcoptes scabiei* infestation in suspected Patients referred to Shahid Beheshti hospital laboratory of Kashan, Iran 2019-2023

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**Background**: Scabies is a pruritic contagious skin disease that caused by *Sarcoptes scabiei* var. hominis. This study was conducted to determine the frequency of *S. scabiei* in suspected patients referred to Shahid Beheshti hospital laboratory of Kashan, Iran 2019-2023.

**Methods:** This cross-sectional study was performed on 84 patients suspected to *Sarcoptes scabiei*. The demographic and clinical symptoms for each of patients were recorded in questionnaire by interview. A deep skin scrap was prepared and wet smear were prepared using 10% KOH. By observation of egg, nymph or adult forms of *Sarcoptes* was identified as positive sample. The data were recorded in SPSS version 16.5 and analyzed by X2 and fisher exact tests.

**Results**: The results of study showed that the prevalence of *S. scabiei* was 18 (21.4%). The rate of infestation in male and females were 7 (20%) and 11 (22.4%) respectively (P = 0.5). The highest rate of Scabies infestation were seen in age group less than 10 years 6 (46.2%) and in the winter 7 (29.2%) respectively. The difference was not statistically significant (P = 0.41, P = 0.71). In this study, out of 18 *Sarcoptes* positive cases, 17 (94.4%) of their families also had disease symptoms. The difference was statistically significant (P = 0.002). Itching and papule were the most clinical signs in positive cases.

**Conclusion:** The results of study revealed that the prevalence of scabies infestation in Kashan, Iran was high. Since the highest rate of infestation was seen in family involvement, health education for increase awareness of family for prevention of disease, along with preventive and control measures is recommended.

Keywords: Prevalence, Sarcoptes scabiei, Kashan

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Prevalence of theileriosis infection in small ruminants in Sistan, Iran

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**Background**: Theileriosis is one of the most common tick-borne diseases that can affect a wide range of domestic and wild animals worldwide. In this study, we determined the infection of theileriosis in the asymptomatic small ruminants by PCR based on the 18S rRNA gene in Sistan region, Iran.

**Methods:** blood samples were collected (August to December 2023) randomly from asymptomatic sheep and goats from 5 districts of the Sistan region including Zabol, Hamoon, Zahak, Hirmad, and Nimrooz. After the DNA extraction with the commercial extraction kit, the quality of the DNA samples was checked with spectrophotometry. The prevalence of the infection was determined using PCR based on the 18S rRNA gene.

**Results**: A total of 11 (22%) from 50 blood samples, were positive for *Theileria* spp. by using PCR.

**Conclusion:** Regarding the findings of this study and previous studies, it is clear that this disease is endemic and economically significant in the region. Identifying specific regional risk factors is essential to mitigate economic losses.

Keywords: Theileriosis, PCR, Sistan

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Oral

Protective immunity induced with a DNA vaccine encoding B- and T-cells multi-epitope SAG1, GRA4 and ROP8 against acute toxoplasmosis in BALB/c mice

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**Background**: *Toxoplasma gondii* infection is characterized by a global prevalence. Constant attempts of scientists have made valuable findings in the development of *T. gondii* candidate vaccines. In this study, DNA vaccine was injected into BALB/c mice, as cocktailed plasmids or as single-gene plasmids to assess the immune response against acute Toxoplasmosis.

**Methods:** BALB/c mice were immunized on days 0, 21, and 42. The immune responses of both vaccinated and control groups were evaluated using cytokine and antibody measurements, lymphocyte proliferation assay and survival time in BALB/c mice.

**Results**: The results indicated that DNA vaccination using multi-epitope SAG1, GRA4 and ROP8 could elicit both cellular and humoral immune responses, and enhanced the survival time in BALB/c mice. In addition, the administration of multi-epitope ROP8, SAG1 and GRA4 could enhance the concentrations of IgG antibody, elicit a mixed IgG1/IgG2a reaction with the predominance of the IgG2a, increase the release of IFN- $\gamma$  cytokine and prolonged the survival time.

**Conclusion:** Here, we report that vaccination using cocktailed plasmids could induce better protective immunity compared to single plasmid for acute *T. gondii* infection

Keywords: Toxoplasma gondii, ROP8, SAG1, GRA4

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Alborz University of Medical, Sciences, Karaj, Iran May 21 - 23, 2024

Oral

Serological diagnosis of cystic echinococcosis: **Challenges and perspectives** 

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Background: The diagnosis of cystic echinococcosis (CE) primarily depends on serological testing and imaging techniques. Numerous serological methods, predominantly based on hydatid cyst fluid and antigen B (AgB), have been developed for the immunodiagnosis of CE. However, their performance has been less than satisfactory.

Methods: Recent studies in serological diagnosis of cystic echinococcosis have been reviewed. The findings of our recent studies in the field of hydatid cyst diagnosis were also taken into consideration, and according to these studies, the limitations and pitfalls of CE diagnosis were determined. Recent advances in the diagnosis of CE were also evaluated and addressed.

**Results**: The use of recombinant antigens has enhanced the performance of serological tests in the diagnosis of CE. In a previous study, we produced two recombinant antigens, B8/1 and B8/2, which demonstrated satisfactory performance in diagnosing CE. These antigens have the potential to be commercialized and utilized for CE diagnosis in our country. Recent studies show that the use of recombinant antigens, especially those that are based on the 8 kDa subunit of the parasite, is promising in the serodiagnosis of CE.

Conclusion: Here, considering the findings of our study, we discuss the pitfalls and challenges in the serological diagnosis of CE, address the limitations of currently available serological tests, and explore the latest developments in the design and application of serological assays for CE diagnosis.

Keywords: Cystic echinococcosis, diagnosis, serological

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Oral

Seroprevalence of toxocariasis in working children and control group in Tehran, Iran

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Background: Toxocariasis, a zoonosis helminthic disease is caused in humans by Toxocara canis and Toxocara cati with worldwide distribution. The disease presents several clinical forms including visceral larval migrans, ocular larval migrans, neurotoxocariasis, and common toxocariasis. In this study the seroprevalence of toxocariasis were investigated in the working children.

Methods: In total, 468 children between the ages of 7-14 years old, including 280 blood samples from working children of Sobh Rooyesh School and 188 samples from control group children were collected from Tehran laboratories. ELISA test was performed to detect IgG antibody against T. canis.

Results: The results of the present study showed that the seroprevalence of toxocariasis was 24 (8.6%) and 10 (5.3%) in the working children and control group respectively. The mean titer of T. canis antibody for working children was higher than the control group and was 26.12 ± 16.99 IU/mL versus 14.83 ± 4.87 IU/mL, which was statistically significant. In this study, the seroprevalence of toxocariasis in girls 18 (13.5%) was higher than boys 6 (4.1%) in working children and the difference between the two groups was statistically significant. Furthermore, a significant relationship was found between factors such as contact with soil.

**Conclusion:** The seroprevalence of IgG antibody against *T. canis* was higher in working children than in the control group in Tehran. The result of the present study indicated a significant difference between working children and the control group in terms of T. canis antibody titer.

Keywords: Seroprevalence, ELISA, toxocariasis, working children

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Seroprevalence of visceral *Leishmanias*is among pregnant women in Jahrom city in Fars province, southern Iran

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**Background**: In this study, the seroprevalence rate and associated risk factors of visceral leishmaniasis (VL) were investigated in pregnant women of Jahrom county, Fars province, southern Iran.

**Methods:** A total of 220 serum samples of pregnant women were assessed for the presence of VL by direct agglutination antigen (DAT). Statistical analyses were performed using SPSS version 25. The associated risk factors were obtained using questionnaires.

**Results**: The overall seroprevalence of VL in pregnant women was 12.72% (28/220). Considering the antibody titer, titer1: 1600 was detected in 23 samples, titer 1:3200 in 4 samples, and titer 1:6400 in one sample. As such, there was a statistically significant difference regarding the age ( $39 \le$  years old with *P*-value: 0.002).

**Conclusion:** We recommend an appropriate health education program for pregnant women and serological screening of VL before pregnancy in endemic cities. Moreover, we believed a need for more epidemiological studies for better understand the status of VL in pregnant women.

**Keywords**: Pregnant-women, seroprevalence, visceral leishmaniasis, Direct-agglutination-test

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Oral

Spatial modeling and risk mapping of vector borne diseases in Iran: A GIS-based survey from 2009-2022

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**Background**: Vector-borne diseases account for more than 17% of all infectious diseases, causing more than 700000 deaths annually. The purpose of this study is to determine the incidence, spatial distribution, and hot spots of vector-borne diseases (Kala-Azar and malaria) in Iran using the GIS analyses during 2009 to 2022.

**Methods:** The data including Vector-borne diseases cases and populations at-risk in different provinces obtained from the Ministry of Health, and Medical Education, Tehran, Iran and other centers from 2009 to 2022. The spatial distribution maps of the Vector-borne diseases were generated. Then, the hot spots of the disease in Iran were determined using spatial analysis of ArcGIS10.5 software. Geographically weighted regression (GWR) analysis in ArcGIS10.5 was used to correlate the temperature, relative humidity, normalized different vegetation index (NDVI) and incidence of vector-borne diseases. Data analysis was performed by linear regression analysis and SPSS 21 software using descriptive statistics test.

**Results**: This study Ardabil, East Azerbaijan, North Khorasan, Fars, Sistan and Baluchistan, and the Bushehr provinces were the hot spots of Vector-borne diseases. In provinces, the highest correlation between humidity, temperature, vegetation density and the incidence of Vector-borne diseases was observed using geographical weighted regression analysis.

**Conclusion:** The use of maps might give accurate estimates of populations at risk. The probability of the presence of Vector-borne diseases in an area was more influenced by climatic conditions such as temperature, humidity, and NDVI. Vector-borne diseases are linked to environmental and climatic conditions.

Keywords: GIS, risk mapping, spatial modeling Correspondence Email(s): Mzeinali\_m@yahoo.com

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<ul> <li>Study on healing of human cutaneous <i>Leishmania</i>sis by using of herbal ointment, Mosh Leish, (a pilot study)</li> <li>Abdolali Moshfe <sup>1</sup> © Ø, Esmaeil Farhadipour <sup>2</sup>, Hossein Sadeghi <sup>3</sup>, Gordafarin Nikbakht <sup>1</sup>, Damoon Razmjouei <sup>3</sup>, Paria Zangane <sup>1</sup>, Nasir Arefkhah <sup>1</sup>, Shahrbanoo Askarian <sup>4</sup></li> </ul>	Study on the prevalence and genetic characteristics based on actin gene sequence analysis in <i>Trichomonas vaginalis</i> isolates among women referred to the gynecology clinics in Motahari and Kowsar hospitals in Urmia	
	Rasool Jafari <sup>1</sup> © Ø, Hadi Sobhani <sup>1</sup> , Tahereh Behroozi-Lak <sup>2</sup> , Marziezh Safari <sup>3</sup> , Shahram Khademvatan <sup>1</sup> , Fatemeh Ramzi	
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<b>Background</b> : Cutaneous leishmanisis is a parasitic disease which is transmitted by sand fly to human, affects lots of people in all over the world that has a great burden on societies and people. Cutaneous leishmaniasis standard treatment is injection of pentavlant antimoniate that has many side effects.	<b>Background</b> : <i>Trichomonas vaginalis</i> is a protozoan of the genitourinary tract of humans and is considered one of the most important non-viral sexually transmitted infections around the world. The current study aimed to determine the prevalence of <i>T. vaginalis</i> infection and its genotype in patients referred to Kowsar and Motabari Hospitals in Urmia	
<b>Methods:</b> In this study 20 confirmed CL patients by observation of <i>Leishmania</i> amastigote in lesion, divided in two group ( $n = 10$ per group). Group one treated with meglumine antimoniate (Glucantime) as control group and group two treated with new herbal ointment 'MOSHLEISH'. Diameter of lesion was measured before and after the treatment and the results were analyzed with chi-score test and Fisher's correction (Fisher's exact test) and Mann-Whitney non-parametric test. <i>P</i> value greater than five percent was considered significant.	<b>Methods:</b> In the present study, 299 vaginal discharges were collected from women referred to Kowsar and Motahari hospitals in Urmia, West Azerbaijan Province from January 2020 to January 2021. The samples were examined microscopically for the presence of <i>T. vaginalis</i> . Then DNA was extracted from the collected vaginal discharges and the parasite's actin gene was amplified by specific primers. The PCR product was sequenced and genotypes were determined by comparing them with the	
<b>Results</b> : Finally, 8 patient of 10 in interventional group were healed completely (80%) and all of 10 patients in meglumine antimoniate (Glucantime) group were treated (100%), which	reference genes in the GenBank. The study was approved by the ethics committee under the ethics code IR.UMSU.REC.1399.177.	
shows that there is no significant difference between the two groups in terms of treatment success ( $P$ 0.05). The mean of lesion's area and their variation was analyzed and no significant differences was seen between two groups after the intervention ( $P > 0.05$ ).	<b>Results</b> : Microscopically 15 (5%) and by PCR 13 (4.37%) patients were positive for trichomoniasis. The genotypes of eight samples could be successfully determined as they were as follows; genotypes G 5 isolates (62.5%), E two isolates (25%), and H one isolate (12.5%).	
<b>Conclusion:</b> This study finally revealed that the new herbal ointment 'MOSHLEISH' has great effect on CL ulcers as same as standard drug choice 'Glucantim' and this trial needs more research with more cases.	<b>Conclusion:</b> The prevalence of trichomoniasis is lower than most of the previous studies in the area, which may be caused by different studied populations or the COVID-19 pandemic period. Also, despite a low prevalence a high genetic diversity of $T$ .	
Keywords: Cutaneous Leishmaniasis, treatment, herbal Ointment	Vaginaris in Orinia was observed.	
Correspondence Email(s): amoshfea@yahoo.com	Keywords: Trichomonas vaginalis, genotype, actin gene	
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Targeting and inhibiting *Plasmodium berghei* growth in Balb/c mice using kojic acid-solid lipid nanoparticles and kojic acid nanostructured lipid carriers

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**Background**: Malaria is a life-threatening infection in the world. The emergence of strains of *Plasmodium* species that are resistant to anti-parasitic drugs, and the lack of licensed high-performance malaria vaccines have raised serious concerns worldwide. In recent years, new treatment strategies such as nanoformulations have been suggested as effective drug delivery.

**Methods:** In this study, kojic acid-solid lipid nanoparticles (KA-SLNs) and kojic acid nanostructured lipid carriers (KA-NLCs) were synthesized using high-speed homogenization and ultra-probe sonication methods to improve their antiplasmodial activities. The obtained nanoformulations were evaluated against the *Plasmodium berghei* malaria parasite in mice. Anti-*Plasmodium* activities and cytotoxicity of the nanoparticles were assed. Fifty percent effective dose (ED50) was calculated as well. Moreover, ex vivo human red blood cells (RBCs) hemolysis was assessed.

**Results**: Kojic acid solution was significantly effective in all concentrations on the seventh day (D7) and the tenth day (D10) (*P*. value 0.05). The toxicity test revealed no toxic impact on the subjects. ED50 was obtained at 150 mg/kg concentration for KA-NLCs and 400 mg/kg concentration for KA-SLNs on D10. The results of the evaluation of KA nanoformulations and KA solution on RBCs indicated that KA nanoformulations could reduce the lysis of RBCs. These results also showed that the lysis of RBCs increased with raising drug concentration in KA nanoformulations, and KA-NLCs (100 mg/kg) gave the least lysis.

**Conclusion:** These results revealed that the KA solution was safe and had no side effects on the subjects in the range of evaluated concentrations. Moreover, the results of this study showed that KA nanoformulations had a better therapeutic effect on *Plasmodium berghei* in Balb/c mice compared with KA solution.

Keywords: Malaria, Plasmodium berghei, kojic acid

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Oral

The advantage and disadvantage of DNA base methods (PCR) in identification of *Leishmania* species, and leishmaniasis

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**Background**: Thirty-seven years have passed since the invention of the PCR machine by American researchers Mullis and Faloona. An invention that created a huge revolution in biology and medicine. In this speech, we would like to express the key role of this evolution in leishmaniasis and our 24 years of experience

**Methods:** Since 1378, research work started with PCR machine. The successful extraction of DNA in the laboratory and the identification of *Leishmania* species, Dermatophytes fungi, *Echinococcus granulosus*, and *Mycobacterium* spp with the Polymorphic DNA Random Amplified method was the beginning of this path. The extraction of DNA from the stained slides for the first time in the world opened the way to identify archival prepared slides in laboratories. Vectors of unknown species of *Leishmania* parasites were identified and the way was opened for extensive research in the field of using different PCR methods such as RFLP, NESTED PCR, MULTIPLEX, and Real Time PCR.

**Results**: However, to what extent can these methods be relied on? What factors does its sensitivity and specificity depend on? How valid is it compared to the standard method of Parasitology? As you know there are millions of sequences in the gene bank, what is the certainty of primers and their specificity?

**Conclusion:** What are the advantages and disadvantages of the genome-based methods? These topics will be discussed based on the experiences gained.

**Keywords**: PCR, *Leishmania*, *Leishmania*sis, advantage, disadvantages

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The effect of the cannabidiol on the lethality of protoscoleces

Abolfazl Miahipour<sup>1</sup> ©, Mahdis Sharifian Zade<sup>1</sup> @, Azadeh Khalili<sup>2</sup>, Mohammad Zibaei<sup>1</sup> of hydatid cyst

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**Background**: Hydatid cyst is a major zoonotic disease and surgery is the best treatment for it. This study was conducted in order to investigate the lethal effect of one of the most well-known cannabinoids of the cannabis plant, i.e. cannabidiol, on protoscoleces of hydatid cyst.

**Methods:** Protoscoleces were extracted from livers infected with hydatid cysts and exposed to different concentrations of cannabidiol (1, 5, and 10  $\mu$ g/mL) for 10, 30, 60, and 120 minutes. The viability of protoscolex was measured by 0.1% eosin staining. Albendazole was used as a standard drug.

**Results**: Cannabidiol in all concentrations used caused significant destruction in protoscolex and showed a direct relationship with increasing concentration (P < 0.05). Albendazole, as a standard drug, caused only  $30.82 \pm 0.5\%$  to disappear after 2 hours, while this amount in the treatment with cannabidiol 5 µg/mL was  $42.52 \pm 0.2\%$  and at the concentration of 10 µg/mL, it reached  $51.04 \pm 0.44\%$ .

**Conclusion:** This study showed that cannabidiol has an acceptable scolicidal effect compared to albendazole and can be suggested as a natural protoscolicidal substance.

Keywords: Cannabis sativa, hydatid cyst, cannabidiol

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Oral

The effects of *Leishmania* RNA virus 2 (LRV2) on proinflammatory biomarkers of *L. major*: an in vitro study on human monocyte cell line (THP1)

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**Background**: Cutaneous leishmaniasis (CL) is a parasitic disease with diverse outcomes. Clinical diversity is influenced by various factors such as *Leishmania* species and host genetic background. The role of *Leishmania* RNA virus (LRV), as an endosymbiont, is suggested to not only affect the pathogenesis of *Leishmania*.

**Methods:** Sample were obtained from CL patients from Golestan province. *Leishmania* species were identified by PCR (LIN 4, 17), and the presence of LRV2 was checked using the semi-nested PCR (RdRp gene). Human monocyte cell line (THP-1) was treated with three isolates of *L. major* with LRV2 and one isolate of *L. major* without LRV2. The treatments with four isolates were administered for the time points: zero, 12, 24, 36, and 48 h after co-infection. The expression levels of pro-inflammatory biomarkers genes including NLRP3, IL18, and IL1 $\beta$ , were measured using quantitative real-time PCR.

**Results**: The expression of the pro-inflammatory biomarkers including NLRP3, IL1 $\beta$ , and IL18 genes in LRV2- was higher than LRV2 + isolates.

**Conclusion:** This finding suggests that LRV2 + may have a probable effect on the *Leishmania* pro-inflammatory biomarkers in the human macrophage model.

Keywords: Leishmania major, Leishmania RNA Virus

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Oral

The larval stages of *Echinostoma* spp. in freshwater snails as the first and second intermediate hosts in Guilan and Mazandaran provinces, northern Iran

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**Background**: Identification of the larval stages of *Echinostoma* spp. in freshwater snails is an essential guide to continue monitoring the possibility of their transmission and the potential of echinostomiasis in areas where trematodes are the primary agent of parasitic diseases. The aim of this study was investigate *Echinostoma* using morphological.

**Methods:** The study was conducted in Guilan and Mazandaran provinces, northern Iran, from April 2019 to October 2021. Overall, 5300 freshwater snails were randomly collected and were identified using external shell morphology. Meanwhile, snails infected with trematodes were studied via shedding and dissecting methods. Larvae stages of *Echinostoma* were identified and the genomic DNA of the samples was extracted. The PCR amplification of the ITSI gene was carried out for 17 isolates and products were sequenced. Seven sequences were deposited in GenBank.

**Results**: Totally, 3.5% of snails containing three species (*Stagnicola, Radix,* and *Planorbis* species) were infected with two types of cercaria, *E. revolutum* with 37 and *Echinostoma* sp. with 45 spines in the collar. Moreover, 35% of the snails were infected with *Echinostoma* spp. metacercaria. Phylogenetic analysis illustrated that isolates were included in two ITSI haplogroups.

**Conclusion:** Conclusion: Results showed the potential hazard of a zoonotic parasite as *Echinostoma* in northern Iran. The potential of disease environmental relationship investigation and resource control optimization is necessary for effective disease.

Keywords: Echinostoma; Cercaria, metacercariae, freshwater snail

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Oral

May 21 - 23, 2024

The prevalence of house dust mites in the homes of individuals with asthma, and comparing it with the control group in the city of Birjand, Iran

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**Background**: Our study aims to evaluate the prevalence of common species of house dust mites (HDM) collected from the homes of asthmatic patients and control group individuals in Birjand city.

Methods: This descriptive-analytical epidemiological study included 100 patients with asthma and 100 clinically healthy individuals. Samples were collected from the living room and bedroom using a vacuum cleaner, and the dust samples were examined directly and through flotation. Subsequently, they subsequently, they underwent molecular confirmation of diagnosis. The data were analyzed using SPSS software version 20. Results: In this study, 100 patients with allergic asthma were examined, with the majority being under 20 years old (35%). The average age of the study participants was reported as  $30.1 \pm 5.1$ years. Regarding gender, there were 35 female adults (35%) and 65 male individuals (65%) included in the study. HDM were found in only 5 samples of the test group (5%) and 1 case in the control group (1%). Morphological and molecular analysis determined that all isolated sample were Dermatophagoides pteronyssinus. Significant statistical differences were reported in terms of the prevalence of Dermatophagoides, type of carpeting, place of residence, and smoking

**Conclusion:** The study revealed a very low prevalence of dust mites among individuals with asthma, possibly due to high temperatures and low annual humidity. Proper control measures are necessary to prevent the growth and reproduction of dust mites in homes to prevent allergic diseases such as asthma.

Keywords: Mite, asthma, Iran, *Dermatophagoides pterisinus* Correspondence Email(s): rahmatsolgi@yahoo.com





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Oral

Ticks analysis for molecular detection and phylogenetic evaluation of stray dogs infecting protozoa from Alborz, Iran

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**Background**: Ticks play an important role in the transmission of parasitic diseases, especially pathogenic protozoa in canine hosts, and it is very important to determine the role and extent of their infection with these pathogens in order to determine important control strategies. This study assessed the molecular prevalence of three protozoan pathogens

**Methods:** A total 300 stray dogs were investigated and 691 ticks (171 male, 377 female and 143 nymph) were detected directly from 45 infested dogs. Species, stage of growth, and gender were determined for each tick. DNA extracted from 224 ticks (26 male, 165 female and 33 nymph). The molecular presence of three protozoan pathogens including *Hepatozoon* spp. (18S rRNA gene), *Leishmania* infantum (kinetoplastid minicircle DNA) and *Babesia* spp. (ssrRNA gene) were investigated using PCR method.

**Results**: One species of ticks, *Rhipicephalus sanguineus* was identified. Two of the target pathogens, *Hepatozoon* spp. (7/83; 8.43%) and Babesia spp. (1/83; 1.2%), were detected by PCR method. Sequence analysis of the ssrRNA gene of detected *Babesia* spp. showed a close relationship to the deposited strains of *Babesia vulpis* in the gene bank.

**Conclusion:** To the best of our knowledge, this is the first study to undertake a phylogenetic analysis of *H. canis* and *Babesia* spp. in stray dogs in Alborz province, Iran and the first report about molecular detection of *Babesia vulpis* from tick infesting dogs in Iran. According to the above results.

Keywords: Tick, dog, protozoa, Alborz, Iran

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Trypanosomiasis of equines in Iran

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**Background**: Trypanosomiasis causes by vector born haemoprotozoa in all mammals such as equines and ruminants. In equines, most common forms are Surra, Malde-caderus, Nagana, Dourine etc. It is a vector borne disease transmitted mechanically by various species of biting flies from the blood of infected animals.

**Methods:** A total of 118 horses blood samples (equestrian horses = 100 and farm horses = 18) from Mazandaran (Amol and Mahmoodabad Hamedan, Amol, Hamadan (Malair and Hamadan), Arak (Komijan) and Lorestan (Borujerd and Rumshkan) were sampled during 2023. Blood samples (up to 5 mL) were collected from the jugular vein into the labeled vacuum tubes containing EDTA for PCR analysis. Giemsa-stained thin blood smears were prepared immediately after peripheral blood collection and further examined microscopically. The DNA extracted from each blood sample was evaluated by molecular methods to determine *Trypanosoma* species.

**Results**: Two positive cases (from equestrian horses) were showed via microscopic observation, which confirmed positive by PCR.

**Conclusion:** Further studies are necessary to distinguish *Trypanosoma* species prevalence and its vectors of other regions in Iran

Keywords: Trypanosoma, horse, Iran

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# May 21 - 23, 2024 Poster

Co-infection of lophomoniasis and pneumocystosis by molecular method in patients with respiratory disorders

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**Background**: *Lophomonas* is an opportunistic pathogen with which human infection is rare. Pneumocystis is one of the major causes of pneumonia in immunocompromised patients.

**Methods:** In a cross-sectional study, 111 BAL samples prepared from patients with respiratory symptoms admitted to the pulmonary department of Imam Reza Hospital in Mashhad were centrifuged.All samples were observed directory for *Lophomonas*, then the PCR test was performed with specific primers for *Lophomonas* and Pneumocystis for all samples taken, and then the data were analyzed.

**Results**: from 111 patients, 48 patients (43.2%) were positive for lophomoniasis infection, 47 patients (42.3%) were positive for Pneumocystosis infection, and 26 patients (23.6%)were diagnosed with co-infection with Lophomoniasis and Pneumocystosis by molecular method. The mean age of patients was 55.27 years. 65 patients were male and 46 patients were female. There was a significant relationship between infection and Lophomoniasis and age groups (P = 0.020), and most of the patients with lophomoniasis were in the age group over 60 years (20 patients). There is a significant relationship between concomitant positive cases of lophomoniasis and pneumocystosis (P = 0.028).

**Conclusion**: According to the results of this study, it is recommended that physicians study the simultaneous infection of lophomoniasis and pneumocystosis in patients with respiratory disorders, which due to the high positive cases obtained in this study can play an important role in early diagnosis, and accelerate the process of appropriate

Keywords: Co-infection, lophomoniasis, pneumocystosis Correspondence Email(s): BerenjiF@mums.ac.ir

# Posters



A study of congenital transmission with a non-virulent *Toxoplasma gondii* isolate obtained from sheep in the mouse model

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**Background**: Toxoplasmosis is an important zoonotic disease that can cause abortion in human and sheep. In the present study, the effect of *T. gondii* strain isolate that was obtained from sheep was examined to produce congenital infection in the mouse model by IFAT and PCR.

**Methods:** Twenty pregnant Swiss breed mice were divided four groups. The groups 1 to 3 were orally infected with 20 sporulated oocysts of *T. gondii* on day 5, 10 and 15 pregnancy, respectively. The group 4 was determined as control negative. The Fertility, litter size and mortality rate were monitored within two generations of consecutive pregnancies.

**Results**: During a three months period of the study, all infected females showed antibodies against *T. gondii*, while the non- infected control mice remained seronegative. There was no difference in the fertility rate of the dams, or in the litter size of infected and control mice. Mortality of offspring of the first and second generations of the infected dams was observed within the first week of life. In this study, the rate of vertical transmission in-group 1, 2 and 3 were 100%, 60% and 50% in first generation, respectively.

**Conclusion**: Based on the results, it is concluded that a nonvirulent *T. gondii* isolate from sheep could be congenitally transmitted through successive generation of mice

Keywords: Congenital transmission, *Toxoplasma gondii*, mouse

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Poster

Cytotoxic effects of Curcumin and Chitosan on *Toxoplasma* gondii Rh strain

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**Background**: *Toxoplasma gondii* is a globally distributed pathogenic parasite. Our study examined the effects of combining curcumin and chitosan on *T. gondii* tachyzoites through in silico, in vitro, and in vivo experiments.

**Methods:** The interaction between curcumin and dihydrofolate reductase (DHFR) was analyzed using a 3D model. Validation of the model was carried out through Ramachandran root-meansquare deviation and VERIFY3D. The cytotoxicity of curcumin and chitosan was assessed via MTT viability assay. Curcumin, chitosan, and a combination of both were administered to BALB/c mice infected with 104 Toxoplasma. The levels of inducible NO synthetase (iNOs), interferon gamma (IFN- $\gamma$ ), interleukin (IL)-5, glutamate oxaloacetic transaminases (SGOT), and glutamic pyruvate transaminase (SGPT) in the serum were subsequently measured.

**Results**: The interaction between curcumin-DHFR and curcumin-DHPS (dihydropteroate synthase) was examined, and the calculated enzyme energy indicated a strong affinity between curcumin and DHFR, while no significant affinity was observed with DHPS. The MTT results of the concurrent treatments showed IC50 rates of 0.1, 0.05, and 0.01 mg/mL at 24, 48, and 72 hours, respectively. In mice treated with curcumin+chitosan, the levels of IFN- $\gamma$ , IL-5, and iNOs were measured at 1.71, 0.51, and 1.51 IU/L, while the levels of SGOT and SGPT were 76 and 84 IU/L, respectively.

**Conclusion**: By combining curcumin and chitosan, the survival time was prolonged by seven days. This combination also modulated the immune response and lessened liver injury, suggesting a promising approach for treating toxoplasmosis.

Keywords: Toxoplasma gondii, curcumin, chitosan, cytotoxicity

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Gel formulation of chitosan-titanium dioxide (TiO2) loaded with glucantime for the treatment of cutaneous leishmaniasis (CL)

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**Background**: In this research, the effectiveness of nanoassemblies made from chitosan-titanium dioxide (TiO2) loaded with glucantime against *Leishmania major* (MRHO/IR/75/ER) investigated in vitro.

**Methods:** The nanoassemblies were incorporated into a 1% Carbopol gel to create chitosan-TiO2-loaded glucantime gel The study involved evaluating the cytotoxic effects of these nanoassemblies on both promastigote and amastigote forms of *L. major* using the MTT assay.

**Results**: The results indicated that the nanoassemblies exhibited higher toxicity against promastigotes compared to amastigotes. After 72 hours of incubation, the 50% inhibitory concentrations (IC50) were  $1.92 \pm 0.4 \mu g/ml$  for promastigotes and  $3.4 \pm 0.5 \mu g/mL$  for amastigotes.

**Conclusion**: Based on these findings, biogenic nanoassemblies of chitosan-TiO2 could serve as a promising therapeutic agent for treating localized cutaneous leishmaniasis lesions.

**Keywords**: Chitosan-titanium dioxide (TiO2), cutaneous leishmaniasis

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Poster

Molecular typing of the actin gene of *Trichomonas vaginalis* isolates in Tehran, Iran

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**Background**: *Trichomonas vaginalis* is a protozoan parasite that causes trichomoniasis with worldwide distribution. This study evaluated actin genotypes of *T. vaginalis* isolates using PCR–RFLP and sequence analysis in Tehran, Iran.

**Methods:** Overall, 850 vaginal samples were collected from women admitted to hospitals affiliated with the Iran University of Medical Sciences in Tehran from 2020 to 2021. The samples were examined by wet mount and cultured. The parasites were harvested, and PCR–RFLP was performed using three endonuclease enzymes of HindII, MseI, and RsaI on *T. vaginalis* isolates. Digestion patterns were then compared, and the genotype of these isolates was defined. The PCR products were sequenced.

**Results**: Overall, 12 (1.4%) isolates of *T. vaginalis* were identified from 850 vaginal samples collected. The most common genotypes were genotype E, seven (58.3%) and genotype G, three (25%), followed by genotype I, two (16.7%), using PCR–RFLP patterns and sequencing. No pattern indicative of mixed infection was found.

**Conclusion**: Genotype E was the most common genotype in the studied group. The phylogenetic analysis indicated the *T. vaginalis* genotype E isolates in a distinct group compared to the genotypes G and I that evolved from a common ancestor

Keywords: Trichomonas vaginalis, actin gene, genotypes

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Sth International & 12th National Congress of Parasitology and Parasitic Diseases of Iran		
NICOPA	Alborz University of Medical, Sciences, Karaj, Iran	
	May 21 - 23, 2024	
Poster	Poster	
Prevalence and genetic characterisation of hydatid cyst in human tissue samples preserved in paraffin from northwest Iran	Prevalence study, morphology and morphometric study, of Gongylonema pulchrum, northern Iran	
Shahram Khademvatan <sup>1</sup> © <b>@</b> , Mahsa Boustani <sup>1</sup> , Elham Yousefi <sup>1</sup> , Farahnaz Noroozinia <sup>2</sup> , Saber Gholizadeh <sup>3</sup>	Tahereh Mikaeili Galeh <sup>1</sup> @, Maryam Nakhaei <sup>2</sup> , Seyed Abdollah Hosseini <sup>3</sup> , Ahmad Daryani <sup>4</sup> , Shahabeddin Sarvi <sup>4</sup> , Shirzad Gholami <sup>5</sup> ©	
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<b>Background</b> : Hydatidosis is caused by the larval stage of <i>Echinococcus granulosus</i> a health issue prevalent worldwide, including in Iran. This investigation was conducted to explore the epidemiological and molecular properties of <i>E. granulosus</i> obtained from hydatid cyst samples in Urmia, a city located in the northwest of Iran.	<b>Background</b> : Livestock parasites, especially in the gastrointestinal tract, have always caused irreparable damage, including death, reduced products, and economic losses. The aim of the present study was to reveal the prevalence, morphological and morphometric study of <i>Gongylonema</i> spp. isolated from the esophagus of slaughtered sheep in Sari, northern Iran.	
<b>Methods:</b> The demographic data of 295 patients with hydatid cysts who underwent surgical procedures from 2010 to 2021 were collected and subjected to statistical analysis in this cross-sectional study. A total of 74 samples were assessed. The DNA extracted from paraffin-embedded hydatid cyst samples was subjected to PCR analysis utilizing mitochondrial genes cox1 and nad1. The PCR products underwent electrophoresis and sequencing, and the obtained sequences were analyzed using BioEdit and BLAST software.	<b>Methods</b> : Esophagi of 340 sheep were collected from Sari's industrial slaughterhouse during Summer and Autumn of 2019 (Summer = 115, Autumn = 225). After longitudinal cutting, the surfaces of the esophageal mucosa were carefully checked under the light. The prevalence of infection was estimated and the morphometric and morphological characteristics of the isolated worms were investigated using a calibrated light microscope and camera lucida, then, the required parts of worm were photographed and measured.	
<b>Results</b> : Out of 295 cases examined, cystic echinococcosis (CE) was notably more prevalent among patients aged 20-30 years ( $n = 24$ ; 1%), villagers ( $n = 70$ ; 17%), and individuals with limited education ( $n = 82$ ; 4%). The group most impacted by hydatidosis was housewives ( $n = 33$ ; 2%), followed by illiterate individuals ( $n = 82$ ; 4%) and farmers ( $n = 17$ ; 3%). The liver ( $n = 52$ ; 78%) and lung ( $n = 40$ ; 12%) were identified as the most common sites for cyst formation. DNA extraction was successful from all 74 paraffinized hydatid cyst samples. Among samples tested using PCR assay with nad1 and cox1 genes, only 27 and 25 FFPE samples were sequenced.	<b>Results</b> : The prevalence of infection was 7.6% (26 isolate), which it was estimated for summer and autumn 12 out of 115 (10.4%) and 14 out of 225 (6.2%), respectively. Comparison of morphological features of nematodes in this study with <i>Gongylonema</i> morphological criteria showed that all of them were <i>G. pulchrum</i> .	
	<b>Conclusion</b> : In order to evaluate the status of <i>Gongylonema</i> , several morphological and molecular studies should be performed on different types of intermediate and final hosts of the parasite in different parts of the country. To increase livestock products and reduce parasitic infections, environmental hygiene and optimal livestock care are essential.	
<b>Conclusion</b> : Molecular findings identified that the G1 strain is the prevailing genotype associated with <i>E. granulosus</i> transmission in northwestern Iran.	Keywords: Gongylonema, prevalence, morphology	
<b>Keywords</b> : Hydatidosis, Iran, sequencing, PCR, frequency <b>Correspondence Email(s):</b> khademvatan@yahoo.com	Correspondence Email(s): sgholami200@gmail.com	



The frequency of intestinal parasitic infection in Urmia, Iran

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**Background**: Despite the considerable advancements in the field of health worldwide, intestinal parasitic infections continue to pose a significant health problem, especially in developing countries like Iran. The present study investigated the frequency of intestinal parasitic infection contamination in patients referred to Dr. Nemati's laboratory in Urmia during 2019-2024.

**Methods:** In this descriptive study, using available data, all the people whose stool samples were tested in Dr. Nemati's laboratory during 2019-2024 were examined. A total of 19117 stool samples have been investigated and tested for intestinal parasites by direct method.

**Results**: Out of 19117 samples, 603 (3.15%) cases (49.75% women and 55.55% men) were infected with intestinal parasites. Of these, *Blastocystis hominis* (80.26%) and *Giardia lamblia* (6.7%) were the most abundant, followed by Entamoeba coli, which constituted 7.13% of them. The rate of infection with *Entamoeba histolytica/dispa*, *Trichomonas hominis*, and *Iodamoeba butschlii* was reported as 4.80%, 1.49%, and 0.99%, respectively. The maximum rate of infection was observed in the age group of (30-50) years. The highest prevalence rate was in the winter season.

**Conclusion**: The study revealed that the most commonly observed parasites among intestinal protozoa were *Blastocystis hominis* and *Giardia lamblia*. Given that, many of these parasites are transmitted through cysts in water and food, promoting health education and adherence to personal and social hygiene standards can mitigate the risk of transmission.

**Keywords**: Intestinal parasites, frequency, infection, Urmia

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Poster

The investigation of the prevalence of pediculosis in Mazandaran province, Iran

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**Background**: Infestation with body lice results in a condition called pediculosis. Lice infestation is one of the most common parasitic infections having a considerable hygienic importance. Therefore, the present study aimed to investigate the prevalence of pediculosis in Mazandaran Province, Iran, from 2012 to 2020.

**Methods:** This descriptive-analytical study investigated the information regarding the frequency of pediculosis and the population of Mazandaran Province, Iran, collected from the health network of Mazandaran province, Iran, from 2012 to 2020. Subsequently, the data were analyzed separately considering gender, age, and location using Poisson regression test.

**Results**: According to the results, there were 243,487 cases of pediculosis from 2012 to 2020 in Mazandaran Province, Iran. The highest level of infection was observed in 2015 (1.61%). Moreover, Pediculosis was 5.6 times more common in females than males (P0.05) and 2.1 times more common in rural areas, compared to urban areas (P0.05). It is worth mentioning that the highest rate of infection was related to the 6-12-year age group.

**Conclusion**: The results show that age, gender, and residential areas are major variables associated with pediculosis. Therefore, it is highly suggested to concentrate on these important variables for the prevention and control of pediculosis.

**Keywords**: Infestation, lice, Mazandaran, pediculosis, prevalence

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	Alborz University of Medical, Sciences, Karaj, Iran
	May 21 - 23, 2024
Poster	Poster
<i>Varroa</i> mite in honeybee colonies in Marvdasht-Fars, southern Iran	Seroepidemiology of <i>Toxocara</i> infection in Isfahan province of Iran
Amin ahmadi <sup>1</sup> ©, Zahra Abhaji <sup>1</sup> , sina Zare <sup>1</sup> ®, Sajad Zare <sup>1</sup>	Gholamreza Pourshahbazi <sup>1</sup> , Elham Hoseini Renani <sup>1</sup> , Hossein Khanahmad <sup>1</sup> , Reza khadivi <sup>1</sup> , Nader Pestehchian <sup>1</sup> , Somayeh Mousayi Mobarakeh <sup>1</sup> .
<sup>1</sup> Biology and Animal Reproduction Science Research Institute, Ardakan University, P.O.Box184, Ardakan,	Mahmood Sadeghi <sup>1</sup> , Hossein Yousofi Darani <sup>1</sup> © <b>D</b>
Iran& Department of Basic Sciences, Faculty of Veterinary Medicine, Ardakan University, Ardakan,	<sup>1</sup> Isfahan University of Medical Sciences
Iran	Background: Toxocara parasites larvae enter the human
<b>Background</b> : <i>Varroa</i> spp. is a significant ectoparasite in honey bees worldwide, potentially leading to a decline in bee colony populations. This research aimed to assess the seasonal prevalence of <i>Varroa</i> mites in apiaries of Maroodasht-Faris province during 2023-2024.	body by ingesting embryonic eggs, which develop on soil. The released larvae from the eggs enter various organs of the body and cause acute or chronic toxocariasis. The epidemiology of this infection is not clear in Isfahan province of Iran.
<b>Methods:</b> A total of 22 hives were randomly selected from various apiaries in Maroodasht-Fars province. Detection of <i>Varroa</i> spp. infestation in adult honey bees was carried out using ether wash, and mites were identified using a diagnostic key (Soulsby, 1982).	<b>Methods:</b> In this cross-sectional work, study population consisted of serum samples of patients referred to clinical labs in Isfahan province. Dot blot method with application of eggs antigen was used for detection of anti- <i>Toxocara</i> antibodies in the sera samples.
<b>Results</b> : The study found a 45.5% prevalence of <i>Varroa</i> spp. infestations in the apiaries.	<b>Results</b> : From 600 examined sera samples 15 (2.5%) were seropositive for <i>Toxocara</i> infection. The highest and the lowest infection rates were from Feredunshahr (6%) and Ardestan (1%) respectively.
<b>Conclusion</b> : Given this high prevalence, the province must implement a consistent control program to reduce infestation rates in the region.	<b>Conclusion</b> : Toxocariasis is a common parasitic disease between humans and animals, which is seen in developed and developing countries. A neglected disease can be cause
Keywords: Prevalence, Varroa app, Hhoney bee	serious symptoms in humans. Parasite larvae do not mature in human body, but they located in different organs such as
Correspondence Email(s): Amin-ahmadi@Ardakan.ac.ir	brain,
	<b>Keywords</b> : <i>Toxocara</i> , human, Isfahan, Iran, seroepidemiology
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	5th International & 12th National Congress of Parasitology and Parasitic Diseases of Iran
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A case report: cystic echinococcosis in neck with G6 genotype

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**Background**: Cystic echinococcosis (CE) is a parasitic zoonosis disease caused by Echinococcus granulosus. In this study, we reported a case of CE in an 11 years old patient.

**Methods:** Hydatid cyst was obtained from a female patient with 11 years-old referred to Mortaz Hospitals, Yazd, Iran. The informed consent and the questioner forms were written from her. The DNA was extracted and amplification was done with the cox1 gene. Sequencing was done for genotype identification. The sequence was submitted in GenBank, NCBI.

**Results**: The hydatid cysts had small size (5 cm). Pathological analysis approved cystic echinococcosis. Amplification showed the expected fragment with the size of 450 bp. Sequencing analysis showed G6 genotype. She had no contact with dogs. In addition, she used the tap water.

**Conclusion**: It seems more awareness and attitude needs in our country as an endemic region.

Keywords: Echinococcus granulosus, Genotypes, Cox1

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Poster

A convenient and sensitive kDNA-PCR for screening of Leishmania infantum latent infection among blood donors in a highly endemic focus, northwestern Iran

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**Background**: Recent global evidence showed that asymptomatic blood donor carriers of *Leishmania* infection would appear as a threat to blood transfusion recipients in endemic areas. Yet, there is no appropriate diagnostic procedure for detecting infection of blood donors in blood banks.

**Methods:** The present study aimed to apply various current diagnostic tests among blood donors in an endemic area of visceral leishmaniasis (VL), Ardabil Province, northwestern Iran. Blood samples were gathered from 860 blood donors in endemic areas of the province between 2017 and 2018, at eight blood donation centers. These samples were assessed using microculture, serological (DAT and rK39-ICT), and molecular-based (conventional kDNA-PCR and HRM-PCR) tests.

**Results**: Of 860 eligible donors, 24 (2.8%) were seropositive for VL by DAT, and 388 (45%) were positive by kDNA-PCR. Moreover, 19 (19/860) were positive for both of them. Out of 19 subjects, 5.3% (1/19) was positive by rK39-ICT, 10.5% (2/19), and 79% (15/19) were detected positive in microculture and HRM-PCR methods, respectively. Nineteen donors were followed up for 2 years, of which 16 (84.2%) had a serological conversion, and 4 (21%) were positive by kDNA-PCR. The sensitivity of kDNA-PCR, and HRM-PCR procedures in detecting *Leishmania* parasite was found to be 98.7%, and 79%, respectively.

**Conclusion**: Our findings justify the use of kDNA-PCR as a convenient and sensitive tool for screening subjects with leishmanial latent infection in blood banks at least in endemic regions. In these areas, however, a PCR-based test should be used to validate *Leishmania* infection among seropositive donors.

**Keywords**: Blood donor, DAT, HRM-PCR, microculture **Correspondence Email(s)**: shabnamdew90@yahoo.com



A deep learning-based model for detecting *Leishmania* amastigotes in microscopic slides: a new approach to telemedicine

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**Background**: The conventional methods employed for detecting the *Leishmania* parasite through microscopy are not only timeconsuming but also susceptible to errors. Therefore, the main objective of this study is to develop a model based on deep learning, a subfield of artificial intelligence that could facilitate automated diagnosis of leishmaniasis.

**Methods:** In our approach for the automatic classification of microscopic images, we employed transfer learning in conjunction with deep learning techniques. Specifically, we leveraged MobileNetV2 as the pre-trained model and subsequently fine-tune it using 292 self-collected high resolution microscopic images (138 positives and 154 negative). In order to mitigate the risk of overfitting, we applied data augmentation in combination with preprocessing layers to preprocess these images. In addition, to ensure reliable results, we adopted the 10-fold cross-validation technique.

**Results**: The final results of utilizing our model for detecting amastigotes in microscopic images are as follows: accuracy of 97.37 $\pm$ 1.42%, specificity of 97 $\pm$ 2.33%, sensitivity of 97.78 $\pm$ 1.81%, precision of 96.77 $\pm$ 2.47%, F1-score of 97.25 $\pm$ 1.46%, and Area Under Receiver Operating Characteristic Curve of 0.9739 $\pm$ 0.0139.Correspondingly, the developed codes and the dataset implemented in this study are available via https://github.com/alrzsdgh/*Leishmania*.

**Conclusion**: The newly devised system is precise, swift, userfriendly, and economical, thus indicating the potential of deep learning as a substitute for the prevailing *Leishmania*l diagnostic techniques.

Keywords: Machine learning, *Leishmania*, microscopic images Correspondence Email(s): mahdifakhar53@gmail.com Poster

A family outbreak of sarcoptis mange in Hamedan, Iran

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**Background**: Identifying the underlying causes of skin diseases is often a challenge in dermatology. The scabies mite, *Sarcoptes scabiei*, is an important, widespread and highly contagious human skin disease. In this article, we describe an outbreak of scabies in a family living in a suburb area of Hamedan in western Iran.

**Methods:** In November 2022, a 21 yo woman was referred to the Arad Medical Laboratory in Hamedan. She complained of incessant itching all over her body, especially at night, for 2 months and after her return from a business trip in Türkiye. She reported that her sister, mother, spouse and colleagues who had accompanied her on the trip had the same symptoms. However, the family's 5yo male ShihTzuterrier dog appeared to be healthy. A superficial skin scraping was performed on the patient, her 14 yo sister and her 43yo mother. The samples were cleared in 10% KOH solution and examined microscopically.

**Results**: One adult female *S. scabiei* was identified in the specimen from the patient, and one *S. scabiei* egg in skin scrape of the family mother.

**Conclusion**: To our knowledge, we report here the second family outbreak of sarcoptic-mange in Iran, the first being an infection of five family members in Esfahan. Application of *S. scabiei* microsatellite markers, which have been shown to be useful in differentiation of varieties is necessary for determining the origin of infestation.

Keywords: Mange, Mite, Scabies

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A label-free electrochemical genosensor based on screen printed gold electrodes with Mxene-Ti3C2/AuNPs/PDA for detection of microsporidia

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**Background**: Microsporidia are an unusually large group of unique, eukaryotic, obligate and intracellular parasites. Microsporidia have been identified as agents of human disease in HIV patient. In this study, a label free electrochemical genosensor was developed for the detection of microsporidia using a probe and a conductive Mxene-Ti3C2/AuNPs/PDA as electrode substrate.

**Methods:** The surface modification of the screen printed gold electrodes (SPGE) was characterized by cyclic voltammetry (CV) and square wave voltammetry (SWV) electrochemical methods, scanning electron microscopy (SEM), atomic force microscopy (AFM), and energy-dispersive X-ray spectroscopy (EDX) analysis. DPV was also used to evaluate the analytical performance of the developed genosensor using 5 mM [Fe (CN) 6]3-/4- as redox mediator.

**Results**: The results obtained by the presented genosensor are compared with those achieved using the Real time PCR. A detection limit of 0.09 fg/ mL-1 was obtain with a linear detection range from 0.1 - 104 pg mL-1.

**Conclusion**: The incorporation of Mxene-Ti3C2/AuNPs/PDA with SPGE improved sensitivity of reaction and reduced assay time, thus providing both simple and fast alternative assay for detection of microsporidia DNA.

**Keywords**: label-free electrochemical genosensor,microsporidia, Mxene-Ti3C2/AuNPs/PDA, SPGE

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Poster

A quick and easy method to investigate the genetic characteristics of *Echinococcus granulosus* from fixed paraffin-embedded tissue samples in human isolates in Sabzvar, Northeast Iran

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**Background**: Since there are ten different genotypes of *Echinococcus granulosus* parasite with different intermediate and final hosts affecting the parasite's life cycle and transmission to humans, and due to the complexity of conventional methods, this study was conducted to determine the genotype of isolated hydatid cysts.

**Methods:** This study used the paraffin tissue samples of patients who underwent surgery which were available in the pathology sample bank of the Vasei and Emdadi Hospitals in Sabzvar city. The DNA content of the samples was extracted after collecting and determining the characteristics using the DNA extraction kit. It was done according to the manufacturer's protocol after performing PCR on the samples and ensuring the presence of the hydatid cyst genome using the special Master Kit. Mix PCR of Solis Biodyne Company and Real-Time device (Bio-Rad) were used, and the genetic identity of hydatid cysts was determined.

**Results**: The present study showed that out of 33 paraffin samples, the DNA of hydatid cysts was not found in 12 samples but 21 samples contained hydatid cyst DNA, two of which were from the brain and 19 from liver tissues. All liver samples were from sheep species (G1), and all brain samples were from buffalo species (G3). Therefore, 9.53% of the *Echinococcus* species collected were buffalo (G3), and 90.47% were sheep (G1).

**Conclusion**: This study showed that *Echinococcus* strains can be identified easily and quickly using high-resolution melting point analysis (HRM) methods based on previous patterns. This method determined that the G1 strain was the dominant strain causing hydatid cyst disease in different human organs, including the liver and brain, in Sabzevar city

**Keywords**: *Echinococcus granulosus*, genotyping, hydatid cyst **Correspondence Email(s)**: Ejavaheri29@yahoo.com



A review on immunity in honey bees

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**Background**: The relevant number of bees in the colonies with high levels of humidity and temperatures of their nests, result in a suitable environment for the development of different microorganisms. In response, they have some social and individual immune systems that protect them from pathogens and some environmental risks.

**Methods:** The behavioral cooperation in the colonies, which is called social immunity, comes from the small tasks of each individual and has an enormous impact on the colony. On the other hand, honeybees have several lines of physical and chemical innate immune defense against pathogens. For instance, several antimicrobial peptides (AMPs) such as abaecin, apidaecin, hymenoptaecin, and defensin, can be found in the hemolymph of honey bees in microbial infections by the activation of hemocyte-mediated cellular immune responses after the binding of Pathogen-Associated Molecular Patterns (PAMP) by Recognition Receptors (PRRs) that trigger signal transduction cascades.

**Results**: Since honeybees are eusocial insects, which are also important pollinators of agricultural crops and other plant species, it is considerable to know that losing honey bee colonies due to different infections has profound ecological and economic implications. As a result, studying different ways of honeybees' defending system seems to be an incredibly important task in order to prevent multiple losses.

**Conclusion**: Discovering various ways of the honeybee's immune system's performances and even manipulating bee gene expressions have been found to be practical in preventing bees' diseases and increasing their health conditions.

Keywords: Honey bee, immune system, health Correspondence Email(s): mpmatila7@gmail.com Poster

A study of the prevalence of gastrointestinal cestodes in golden jackals in northern, northeastern, and northwest Iran

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**Background**: There are 194 species of parasites reported from golden jackals across their geographical range. There is a wide variety of parasites in this species, because of their wide geographical range, territorial mobility, and unselective diet. It is common for domestic dogs and cats to carry these parasites.

**Methods:** From 2019 to 2022, 69 golden jackals, which were killed due to car accidents or illnesses, were collected. To verify the presence of Cestodes, morphology and morphometry analyses were performed.

**Results:** In this study, 71.4 % of golden jackals were found infected with Mesocestoides, followed by *Dipylidium caninum* (5.7%), *Echinococcus granolusus* (5.7%), and *Taenia hydatigena* (5.7%). Approximately 8.5% of golden jackals had double infections with *Mesocestoides* and *T. hydatigena*, while 2.85% had triple infections with *Mesocestoides* and *T. hydatigena* and *E. granolusus*.

**Conclusion**: The most common tapeworms found in golden jackals are *Mesocestoides* spp., *Taenia* spp. *D. caninum* and *Echinococcus*. Some species of cestodes have a significant impact on zoonotic transmission (i.e. *E. granulosus*) and others with a minor impact (i.e. *D. caninum* and *Mesocestoides*). The most commonly reported species of *Taenia*.

**Keywords**: Golden jackals, *Mesocestoides*, *Dipylidium*, *Echinococcus* 

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A survey of strongyloidiasis in immunodeficiency patients referred to hospitals of Mazandaran province simultaneous use parasitological, serological and molecular techniques between 2021 to 2022

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**Background**: Strongyloidiasis is an intestinal nematode caused by *S. stercoralis*, one of the neglected tropical diseases. This study aimed to investigate *S. stercoralis* in high-risk target groups with simultaneous use of parasitological, serological and molecular techniques in northern Iran.

**Methods:** This study was performed on patients suffering from 92 autoimmune disorders including 40 cancer undertaking corticosteroid treatment or chemotherapy and 52 HIV+ patients. Patients were examined using direct smear examination, agar plate culture, and ELISA test. Since agar plate culture was considered the reference diagnostic test, culture-positive samples were confirmed by PCR amplification and the sequencing of the Cox1 gene.

**Results**: Serological investigation was showed 4 Out of 52 HIV+ (7.69%) and 15 out of 40 cancer patients (37.5%) were infected with *S. stercoralis*. Out of the 92 patients in the study, stool microscopy for Strongyloides rhabditiform larvae was positive for 4 patient. The stool samples of these patients were negative in agar plate cultures. Using nested PCR, 4 samples (4.34%) were found positive for *S. stercoralis*.

**Conclusion**: The study emphasizes the need for screening highrisk patients for Strongyloidiasis, especially in endemic areas; it is advised that all high-risk patients be screened for *S. stercoralis* infection before beginning medication, as the ELISA test had the highest frequency and recommends aspiration from the duodenum due to its high prevalence.

Keywords: Strongyloidiasis, immunodeficiency, ELISA, PCR, Mazandaran Correspondence Email(s): hosseini4030@gmail.com Poster

A systems biology approach to immune response biomarkers of *Heligmosomoides polygyrus* infection in rodents

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**Background**: *Heligmosomoides polygyrus* is an intestinal nematode in rodents. This roundworm is found in the lumen and lymph nodes and causes changes in the immune response. However, the molecular mechanisms of *H. polygyrus* infection are not fully understood. This study explores to recognize upregulated genes in lymph nodes compared to duodenum.

**Methods:** At first, the microarray profiling dataset related to gene expression in duodenum and lymph nodes of mice was obtained from gene expression omnibus (GEO) database. In addition, GEO2R analyzer tool was used for differentially expression genes (DEGs). Next, gene ontology (GO) and pathways enrichment conducted by DAVID database. Also, Search Tool for the Retrieval of Interacting Genes (STRING) was employed for protein-protein interaction (PPI) of DEGs. Finally, hub genes obtained by Cytoscape.

**Results**: Of 349 recognized DEGs, 107 and 242 were upregulated and downregulated genes, respectively. The GO enrichment on upregulated genes in DAVID revealed the biological processes like immune system process and adaptive immune response. The database of Kyoto Encyclopedia of Genes (KEGG) was used to confirm the enriched pathways, and pathways including Th17 cell differentiation, Th1 and Th2 cell differentiation, and Hematopoietic cell lineage were obtained. Finally, the upregulated genes in lymph nodes analyzed by Cytoscape plugins and hub genes (Cd27, Cd3e, Sell, Cd40, Ccl5, Lck, Cxcr5, Ccr6, Cd79b, Cd22) were recognized.

**Conclusion**: Overall, these genes identification shows useful information about the mechanisms involved in the immune and lymphatic system when faced with parasitic infections. In addition, lymph node hub genes can be a potential biomarker for the design of vaccines against parasitic infections and related immune responses in future studies.

**Keywords**: Bioinformatics, *Heligmosomoides polygyrus*, Biomarker, network

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Ability of *Toxoplasma gondii* excreted/secreted antigens to discriminate between low and high IgG avidity antibodies: usefulness for diagnosis of acute toxoplasmosis

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**Background**: The immunoglobulin G [IgG] avidity test discriminates between chronic and acute toxoplasmosis. The aim of the present study was to evaluated the discrimination of acute and chronic toxoplasmosis by IgG avidity test using Excretory/Secretory Antigens (ESAs) of *Toxoplasma gondii*.

**Methods:** This study was performed in the Rafsanjan University of Medical Sciences, Rafsanjan, Iran. Blood samples were collected from *T. gondii*-infected inbred Fischer rats, and their IgG avidity was measured using ESAs prepared from cell-free media.

**Results**: The percentage of Avidity Index [AI] in the sera samples (n = 7) collected 20 and 30 days after infection significantly increased (P > 0.05) compared to those collected after ten days of infection.

**Conclusion**: Acute toxoplasmosis was discriminated from chronic toxoplasmosis, based on IgG avidity. ESAs can be used to detect anti-ESAs IgG avidity to discriminate acute toxoplasmosis.

**Keywords**: Excretory secretory antigens; IgG avidity;

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Poster

An integrated bioinformatic analysis of microarray datasets to identify biomarkers and miRNA-based regulatory networks in leishmaniasis

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**Background**: Micro RNAs (miRNAs, miRs) and relevant networks and signalings might exert crucial functions in pathogenesis process of leishmaniasis infection. Thus, the aim of this study was to bioinformatic analysis of microarray datasets to identify pivotal biomarkers and miRNA-based regulatory networks in leishmaniasis.

**Methods** We conducted a transcriptomic analysis by employing microarray gene expression profiling to identify the key genes and miRNAs that are involved in the regulation of leishmaniasis. The gene expression profiles of healthy controls with those of individuals infected with each of the four *Leishmania* species including *L. mexicana*, *L. major*, *L. donovani*, and *L. braziliensis* were compared. We conducted enrichment analysis by utilizing Enrich database. Additionally, we have constructed a protein-protein (PPI) network to identify the hub genes and the prognostic value of these hub genes was evaluated. The miRNAs that interact with these hub genes were identified using some online tools.

**Results**: Differentially expressed genes (528) were identified and enriched in various biological processes. The hub genes of datasets were identified accordingly; GSE42088 (SOCS3, TNFAIP3, JUN and, TNF), GSE69252 (CCR7, IDO1), GSE55664 (CXCL8, CXCL9, and CXCL10), GSE63931 (CXCL9, CXCL10, and FCGR3A), GSE64610 (VEGFA, IL1B and TNF), GSE43661 (LRRK2, HSPA1B and, RPL13). TNF, SOCS3, JUN, TNFAIP3, and CXCL9 exhibit prognostic value, serving as potential biomarkers for leishmaniasis. MiRWalk data revealed a significant interaction of hsa-miR-8085, hsa-miR-4673, hsa-miR-4743-3p, hsa-miR-892c-3p, hsa-miR-4644, hsa-miR-671-5p, hsa-miR-7106-5p, hsa-miR-4267, hsa-miR-5196-5p, and hsa-miR-4252 with the majority of the hub genes, suggesting such miRNAs play function in the development of leishmaniasis.

**Conclusion**: Our research clarified novel perspectives on the gene and miRNA-based regulatory networks in leishmaniasis. The hub genes and hub miRNAs identified in this study could be potentially suggested as therapeutic targets or biomarkers for the management (treatments and diagnosis) of leishmaniasis.

Keywords: Leishmaniasis, regulatory network, miRNA, microarray

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May 21 - 23, 2024

#### Poster

Anti leishmanial effects of Crocin on *Leishmania major* (MRHO/IR/75/ER) in vitro and in vivo

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**Background**: Leishmaniasis is a zoonotic disease caused by the *Leishmania* parasite. Due to therapy limitation, the development of studies focused on the proper use of drugs to discovery of new antileishmanial compounds is required. Therefore, this study is designed to investigate the anti-leishmanial effect of crocin invitro and invivo.

**Methods** Crocin were extracted from the aerial parts of *Crocus* sativus. The promastigotes of *Leishmania major* were prepared and cultured in RPMI. For in vitro study, the promastigotes were added to the 96-well plate and 100  $\mu$ l of each extraction were then added. After incubation time, the numbers of viable promastigotes were assessed using eosin 0.1 % as vital staining. For in vivo study, after the presence of the lesion in the bases of the tail of Balb/c mice, treatments were done daily for 28 days and the size of lesion were measured in Day 0, 7, 14, 21 and 28.

**Results**: In vitro anti-leishmanial effect of all concentrations was increased during different times and the highest rate was observed at concentration of 100 mM after 48 h. In in vivo, the mean of lesions size significantly decreased in the group treated with the crosin compared to the control groups, during time and the highest reduction in wound size was related to the concentration of 100 mM after 28 days of treatment.

**Conclusion**: Crocin showed a significant anti-leishmanial effectiveness against *L. major* in vitro and in vivo and investigated synergistic effects with Amphotericin B in the treatment of cutaneous leishmaniasis.

Keywords: Cutaneous leishmaniasis, Amphotericin B, Crocin

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Poster

Anti-Leishmania major effect of Calotropis procera extract on promastigotes and amastigotes invitro

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**Background**: *Leishmania*sis, a tropical disease caused by protozoan parasites belonging to the genus *Leishmania*, is often overlooked despite its high global incidence. This particular study aimed to assess the impact of *Calotropis procera* (Asclepiadaceae) extract on the viability of *Leishmania major*'s promastigotes and amastigotes through in vitro experiments.

**Methods:** A methanol maceration method was employed to prepare the extract from the leaves of *C. procera* seedlings. The growth-inhibitory effect of the extract on promastigotes was assessed using the colorimetric cell viability assay, specifically the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Furthermore, the level of ROS in promastigote cultures was determined using the 2',7'-dichlorofluorescein diacetate (DCFH-DA) method after treatment with the extract, and compared to untreated cultures (control). Additionally, the expression levels of TNF- $\alpha$ , IFN- $\gamma$ , and inducible iNOS genes were determined in peripheral blood mononuclear cells (PBMCs) infected with *L. major*, following exposure to the extract, and compared to the control.

**Results**: The MTT assay results indicated that the C. procera extract significantly hindered the proliferation of *L. major* promastigotes, displaying IC50 values of 377.28 and 222.44  $\mu$ g/mL for 24 and 72 hours, respectively (p 0.01). Upon exposure to 222.44 and 377.28  $\mu$ g/mL of C. procera extract, ROS production in *L. major* promastigote cultures increased by 1.2- to 1.65-fold and 2- to 4-fold, respectively, in comparison to the control (p 0.05). Furthermore, the C. procera extract triggered substantial elevations in the gene expression of TNF- $\alpha$ , IFN- $\gamma$ , and iNOS in infected PBMCs as opposed to the control (p 0.01).

**Conclusion**: Based on its anti-leishmanial activity, *C. procera* can be considered a novel plant source for potentially treating leishmaniasis.

Keywords: Amastigote, Calotropis procera, Leishmania major

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Sth International	& 12th National Congress of Parasitology and Parasitic Diseases of Iran
	Alborz University of Medical, Sciences, Karaj, Iran
	May 21 - 23, 2024
Poster	Poster
Anti-Leishmania major effect of sanguinarine and nano chitosan on promastigotes and amastigotes invitro	Anti-leishmanial effect of the hydroalcoholic extract of the leaves, roots and seeds of <i>Arctium lappa</i>
Elham Yousefi <sup>1</sup> © @, Shahla Amani <sup>1</sup> , Shahram Khademvatan <sup>1</sup> , , Behzad Jafari <sup>1</sup> , Negar Asadi <sup>1</sup> , Vahid Shafiei-Iranneiad, <sup>2</sup> , Gordon S, Howarth <sup>3</sup>	Sedigheh Saberi <sup>1</sup> © <b>D</b> , Elham Maleki <sup>2</sup> , Afsaneh Yegdaneh <sup>3</sup> , Sakineh Akbari <sup>4</sup>
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<b>Background</b> : The main aim of this study was to evaluate the anti- leishmanial properties of sanguinarine and nano chitosan (CS NPs) against <i>Leishmania major</i> , with a potential mechanism involving the regulation of TNF- $\alpha$ and iNOS gene expression. To identify the	<sup>4</sup> Department of Parasitology and Mycology , School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
potential targets for sanguinarine, the energy-minimized structure was docked using AutoDock 4.3	<b>Background</b> : Zoonotic cutaneous leishmaniasis is a common and endemic infectious disease in many parts of the world and Iran. Due to <i>Arctium lappa</i> wide therapeutic applications, the anti-leishmanial effect of the hydroalcoholic extract of its leaves roots and seeds has
<b>Methods:</b> Utilizing the colorimetric MTT assay, the viability of <i>L.</i> <i>major</i> promastigotes was determined following exposure to different concentrations of sanguinarine and CS NPs in a	been investigated in this research.
concentration- and time-dependent manner. The levels of reactive oxygen species (ROS) were evaluated in the treated promastigotes. Expression of TNF- $\alpha$ and iNOS was analyzed in PBMCs infected with sanguinarine and CS NPs at 24, 48, and 72 hours post-treatment. The interactions between sanguinarine, PTR1, and ARG-L, along with the calculated energy of these enzymes, revealed a strong binding affinity of sanguinarine towards both	<b>Methods:</b> The leaves, seeds and roots of the greater burdock plant were extracted. In the in vitro phase, its cytotoxic effects, anti-leishmanial effects on promastigote and amastigote forms were investigated and indexed, allowing for IC50 and CC50 determination. In the in vivo stage, after preparing the leishmaniasis mouse model in 55 BALB/c mice were dosed with concentrations of 50, 100, 200 mg/kg, wounds were sized and the liver and spleen parasite burden was checked.
<b>Results</b> : After 24 and 48 hours, the IC50 values of sanguinarine combined with 10% CS NPs were determined to be 124.76 $\mu$ M and 89.25 $\mu$ M, respectively. Following a 24-hour exposure, Sanguinarine-CS NPs significantly increased the expression of TNF- $\alpha$ and iNOs in <i>L. major</i> -infected PBMCs (p 0.05).	<b>Results</b> : The results of this study illustrated a correlative time- dependent administration of hydroalcoholic extract of leaves, roots, and seeds of the <i>Arctium lappa</i> significantly reduced the growth of parasites in vitro ( $P = 0.001$ ). In the In vivo phase, a significant difference was observed between the mean diameter of the wounds before and after treatment in the 200 mg/kg concentration groups of the extracts of the root, leaves and seeds ( $P = 0.05$ ).
<b>Conclusion:</b> Sanguinarine-CS NPs hold great potential as an effective anti-leishmanial agent. Additionally, the combination of Sanguinarine and CS NPs effectively suppressed the proliferation and viability of <i>L. major</i> promastigotes, while also modulating the expression of TNF- $\alpha$ and iNOS and increasing ROS levels	<b>Conclusion</b> : The investigation established that hydroalcoholic extract of the leaves, roots, and seeds of the greater burdock in high concentrations exhibited beneficial inhibitory effects on the leishmanial lesions, which may be attributable to the proven anti- inflammatory, antimicrobial and immunomodulatory properties of the naturally present compounds found within the extract such as
Keywords: Anti-Leishmania, nano chitosan, sanguinarine	Keywords: Anti-leishmanial, BALB/c mice, Arctium lappa
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Anti-leishmanial effects of a novel biocompatible non-invasive nanofibers containing royal jelly and propolis against Iranian strain of *Leishmania major* (MRHO/IR/75/ER): an in vitro study

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**Background**: Current medications especially the pentavalent antimonial compounds have been used as the first line treatment of cutaneous leishmaniasis (CL), but they have limitations due to serious side effects such as drug resistance, cardio and nephrotoxicity, and high costs. Hence, the demand to find more usable drugs is evident.

Methods: By electrospinning method, a new type of nanofiber were synthesized from royal jelly and propolis with different ratios. Nanofibers were characterized by Scanning Electron Microscope (SEM), Transmission Electron Microscopy (TEM), Thermogravimetric Analysis (TGA), Contact angle, and Fouriertransform infrared spectroscopy (FTIR). The Half-maximal inhibitory concentration (IC50), Half-maximal effective concentration (EC50) and the 50% cytotoxic concentration (CC50) for different concentrations of nanofibers were determined using quantitative calorimetric methods. Inductively coupled plasmaoptical emission spectrometry (ICP-OES) and flow cytometry were performed as complementary tests.

**Results**: The results showed that the proposed formulas provide a new achievement that, despite the significant killing activity on *L. major*, has negligible cytotoxicity on the host cells. Royal jelly nanofibers have significantly shown the best 72 hours results (IC50= 35  $\mu$ g/ml and EC50=16.4  $\mu$ g/mL) and the least cytotoxicity.

**Conclusion**: This study presents a great challenge to introduce a new low-cost treatment method for CL, accelerate wound healing, and reduce scarring with minimal side effects and biocompatible materials. Royal jelly and propolis nanofibers significantly inhibit the growth of *L. major* in-vitro.

Keywords: Leishmania major, nanofiber, propolis, royal

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Poster

Anti-parasitic activity of CM11 against trophozoites of *Trichomonas vaginalis* 

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**Background**: Trichomoniasis is a non-viral STI caused by the protozoan parasite *Trichomonas vaginalis*. Metronidazole is the standard treatment for this infection. Although this treatment has been successful, it is often related to severe side effects and the failure of treatment in resistant trophozoites.

**Methods:** For in vitro experiments, Hela cells were treated with different concentrations of CM11 (1-32  $\mu$ g/ml) compared to metronidazole (MET) (1.25-80  $\mu$ g/ml). MTT assay evaluated the cell viability (CC50). Then, the inhibitory concentration (IC50) values were determined for treating trophozoites of *T. vaginalis*.

**Results**: CM11 was effective on trophozoites of *T. vaginalis* and had a time and dose dependent manner. The results of the MTT assay showed that the CC50 values of CM11 and MET were estimated at 7.03 µg/ml and 21.34 µg/mL after 24-h, respectively. The inhibitory concentrations (IC50) of CM11 and MET for G and I strains were estimated at (G: 3.7 and I: 2.7 µg/mL) and (G: 0.6 and I: 0.35 µg/mL) after 24-h, respectively.

**Conclusion**: Antimicrobial peptides could be applied as promising anti-*Trichomonas* compounds for treating trichomoniasis.

**Keywords**: *Trichomonas vaginalis*, antimicrobial peptides, metronidazole

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Antigen B modulates anti-inflammatory cytokines in the EAE model of multiple sclerosis

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**Background**: Multiple sclerosis (MS) is characterized by the destruction of the blood–brain barrier, loss of myelin sheath, and contribution of inflammatory interleukins such as TNF-alpha, interleukin-17, and interleukin-6.

**Methods:** The current study investigated the effect of antigen B of hydatid cyst fluid on the reduction of anti-inflammatory cytokines and nerve conduction velocity in rats with experimental autoimmune encephalomyelitis (EAE)-induced MS. After isolation of antigen B from sterile cyst fluid, the rats were randomly divided into four groups: saline, EAE, EAE + teriflunomide (EAE + TF), and EAE + antigen B (EAE + AngB). The EAE model was induced using cow spinal cord homogenization, in combination with Freund's complete adjuvant. The serum concentration of cytokines including IL-1B and IL-17, IL-10, IL-6, and TNF-X was measured by the ELISA method,

**Results**: Nerve conduction velocity and IL-10 concentration were increased in the antigen B group. The results of this study showed that antigen B reduced the inflammatory component of the EAE MS animal model by modulating the immune system compared to teriflunomide, which eventually led to a reduction in symptoms at the behavioral and electrophysiological level.

**Conclusion**: It seems that antigen B plays a critical role in regulating immunity and it can be used as a possible therapeutic agent to modulate the immune system in MS patients. It might be rational to consider hydatid cyst fluid antigen as a modifier in MS.

Keywords: EAE, cytokines, multiple sclerosis, antigenB

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May 21 - 23, 2024

Antigen B polymorphism and Genetic diversity in Echinococcus isolates of Echinococcus granulosus in northeastern Iran

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**Background**: Comparing the diagnostic potential of *Echinococcus* granulosus antigens has shown that antigen B2 has better diagnostic power due to high genetic diversity in different hosts. This study aims to investigate the genetic polymorphism of antigen B2 among different isolates of *E. granulosus* in northeastern Iran. **Methods:** Hydatid cyst samples including 50 sheep cysts, 10 cattle cysts, 15 camel cysts, and 15 human cysts were collected from slaughterhouses and hospitals in northeastern provinces. Then DNA was extracted from the germinal layer of cow samples and protoscolexes of other samples. During the PCR reaction, the samples were amplified with the B2 antigen-specific primer and PCR-RFLP was used to evaluate the AgB2 polymorphism in the isolates. AluI & EcoRI restriction enzymes were used to cut the created bands.

**Results**: A 380 bp band was observed in all isolates except cattle isolates. When cutting the bands obtained from these samples with the AluI enzyme, the same bp pattern (270 and 110) was observed, but no cutting was observed with the EcoRI enzyme. The same pattern observed in different samples indicates the intra-strain genetic similarity of B2 antigen in sheep, camel, and human isolates. Cattle samples were not amplified with B2 antigenspecific primer, which indicates the interstrain genetic diversity of B antigen in cow isolates.

**Conclusion**: The genetic diversity of antigen B in different isolates of *E. granulosus* in endemic areas should be investigated to design and standardize tests that use this antigen to detect specific human antibodies.

**Keywords**: *Echinococcus granulosus*, antigen B, genetic diversity

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Anti-leishmanial activity and mechanisms of action of azacitidine alone and along with meglumine antimoniate on *Leishmania major* 

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**Background**: Leishmaniasis is a human-animal protozoan disease prevalent in most parts of the world. Leishmaniasis has no effective vaccines, and the accessible first and second-line treatment selections alone or dual are insufficient. This study aimed to explore the in vitro and in silico anti-leishmanial activity of azacitidine (AZA) on *L. major*.

**Methods:** The in silico method was used to assess the possibility of the interaction of AZA into the binding pocket of inducible nitric oxide synthase (iNOS), a leading defensive oxidative metabolite. Next, in vitro anti-promastigote, and anti-amastigote activity of AZA was determined using an MTT assay and a macrophage model, respectively. Cytotoxic effects of AZA and meglumine antimoniate (MA) were also assessed by MTT assay on murine macrophages. All experiments were done in triplicate.

**Results**: The results displayed that AZA interacted with Ser133, Gln134, and Lys13 amino acids of iNOS, and the molecular docking score was obtained at -241.053 kcal/mol. AZA in combination with MA significantly inhibited the growth rate of nonclinical promastigote (IC50 247.6  $\pm$  7.3  $\mu$ M) and 8.5-fold higher of clinical intramacrophage amastigote stage (29.8  $\pm$  5.3  $\mu$ M), compared to the untreated group. A significant upsurge of Th1 subsets and transcription genes and a meaningful decline in Th2 cytokines subclasses at the equivalent concentrations of AZA and MA was observed. The apoptosis of AZA along with MA was significantly induced on *L. major* in a dose-dependent manner.

**Conclusion**: This study demonstrated that AZA in combination with MA has a potent anti-leishmanial mechanism, promoting immune response and enhancing an immunomodulatory role toward the Th1 pathway. This study is a basic study for applying more awareness about the mechanisms of AZA along with MA in animal models in the future.

Keywords: Anti*Leishmania*l, Azacitidine, Meglumine antimoniate, *Leishmania* Correspondence Email(s): mehdimorovat@ymail.com Poster

Anti-leishmanial and immunomodulatory properties of diallyl sulfide and meglumine antimoniate on *Leishmania infantum*: multiple experimental assays, and transcriptomic analysis

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**Background**: Leishmaniasis is considered an important tropical disease that has no sufficient therapeutic modality. This study aimed to assess Diallyl sulfide (DAS), a dynamic component of garlic and meglumine antimoniate (MAT) as a standard drug using experimental models and transcriptome analysis against *Leishmania infantum* stages.

**Methods:** Multiple approaches were performed to monitor and evaluate the anti-*Leishmania*l properties of these drugs on promastigotes and amastigotes using the colorimetric method, apoptotic profile by flow cytometry, differently expressed cytokine genes related to M1 and M2 macrophages, and also, changes in the amount of reactive oxygen species (ROS). In addition, expression profiling and differential gene expression analyses were carried out for DAS and MAT using the Next Generation Sequencing (NGS) technology. Gene Ontology analysis and Gene Pathway analysis were performed using "Blast2Go" software, "DAVID" and "Reactome.org" databases, respectively.

**Results**: The results of this study showed that examining the selectivity index (SI) of two drugs demonstrated a safe mixture. Also, this combination promoted an immunomodulatory mode of action toward M1-Th1 to eliminate the parasite and increase the amount of ROS. Furthermore, the finding presented that multiple pathways up-regulated are associated with signaling networks, such as "putative peptidase and PHB domain" transcript.

**Conclusion**: This research illustrates the importance of using complementary technologies to elucidate and appreciate DAS and MAT in contributing to the leishmaniasis treatments. Identification of the upregulated and downregulated transcripts, biological process, molecular function, cellular component and apoptotic pathways deserve the development of new strategies to treat the leishmaniasis effectively.

Keywords: Leishmaniasis, diallyl sulfide, transcriptome, apoptosis

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Anti-malarial nano-drug delivery system based on graphene quantum dot on *Plasmodium falciparum*: Preparation, characterization, toxicological evaluation

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**Background**: Malaria infects millions of people every year. Emerging drug resistance in many strains of *Plasmodium falciparum* against the currently used antimalarial is one of the major obstacles in the way of elimination of falciparum malaria infection.

**Methods:** Sodium hydroxide, citric acid, methanol, ethanol and Giemsa stain were obtained from Merck. Artesunate (Art) (MW = 384.42, cat No. A3731), Mefloquine (Mef) (MW = 414.77, EC No. 257-412-0, MDL No. MFCD00797519), Gentamicin, sorbitol and Dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich. Packed red blood cells O+ (PC), Fresh frozen plasma AB+ (FFP) were obtained from Blood Transfusion organization of Iran. Normal Saline Solution was purchased from Iranian parenteral and pharmaceutical co. RPMI 1640 Medium 1640 (1×) + GlutaMAX liquid was obtained from Gibco. Albumax were purchased from Vizcarra pharma (Vizcarra Pharmaceutical, Ermita, Manila, Philippines).

**Results**: The FESEM and TEM images of GQD are presented in Fig. 1A and B. The GQDs are well dispersed with a uniform size distribution, whose average sizes are about 6.53 nm (insets in Fig. 1). TEM images also show crystalline GQDs with a lattice measurement of 0.203 nm, which coincide with the graphene (002) plane [26]. The images with a hexagonal honeycomb structure demonstrate that the GQDs are nearly defect-free graphene single crystals [27]. Numerous literature reports signify the nanoparticles to be efficient peroral drug delivery systems. However, their efficient uptake is evidently charge and size-dependent with smaller nanoparticles generally.

**Conclusion:** Malaria is one of the most important parasitic infections in the world. More than 40 % of the world's population lives in malaria-endemic areas. Due to the emergence of *P. falciparum* strains resistant to chloroquine, mefloquine, and artesunate in recent decades, developing countries facing difficulties in treating acute cases.

Keywords: Artesunate, graphene quantum, dot malaria

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Poster

Apoptosis in *Echinococcus granulosus* protoscoleces induced by FLBZ-loaded mPEG-PCL nanoparticles

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**Background**: Despite the use of various benzimidazole compounds for the treatment of human echinococcosis, none of the existing drugs is able to effectively treat the disease. The purpose of this study was the evaluation of apoptosis induction in E. granulosus protoscoleces by flubendazole-loaded mPEG-PCL (FLBZ-loaded nanoparticles).

**Methods:** The viability percentage of protoscoleces was determined under the light microscope and the methylene blue dye exclusion test. The TUNEL (terminal deoxy nucleotidyl transferase dUTP nick end labeling) method was used to investigate the apoptotic protoscoleces. Protoscoleces were fixed after 24 and 48 hours of incubation with FLBZ-loaded nanoparticles and free FLBZ at the final concentrations of 5 and 10  $\mu$ g/mL in 2% paraformaldehyde, 2.5% glutaraldehyde and then operated according to the method of the apoptosis detection kit (In Situ Cell Death Detection Kit), and the nuclei were stained with fluorescent stain, 4',6-diamidino-2-phenylindole (DAPI).

**Results**: After 24 hours of incubation, there was no significant difference in the amount of apoptosis in any of the studied groups compared to the control group, but after 48 hours of incubation, there was a significant difference between the control group and both groups of FLBZ-loaded nanoparticles (with both concentrations of 5 and 10 µg/mL) and free FLBZ (with a concentration of 10 µg/mL) (P < 0.05).

**Conclusion**: Although the increase in the percentage of apoptotic protoscoleces in the group of FLBZ-loaded nanoparticles was much higher and more significant than that of free FLBZ, no significant difference was observed between the two groups. Therefore, FLBZ-loaded nanoparticles are capable of causing apoptosis in the nuclei of protoscoleces.

Keywords: Echinococcus granulosus, protoscolex, nanoparticles, apoptosis

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Application of HRM (High-Resolution Melting) in the detection of intestinal protozoa

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**Background**: Intestinal protozoa are found throughout the world, thereby presenting substantial challenges in their detection the technique used for the detection of protozoans is the HRM technique. The objective of this study was to examine the application of HRM in articles about the diagnosis of protozoa.

**Methods:** In this review study, articles related to Application of HRM High-Resolution Melting in the detection of Intestinal protozoa were reviewed in various databases of Web of science, Pubmed, Scopus, Google Scholar and etc from 2018 to 2024.

**Results**: A study conducted in 2022 in Birjand, among 1800 stool samples, 58 samples were positive for Giardia parasite and by HRM method, 91.36% group A and 8.62% group B were identified. In a study conducted in Tehran in 2021, 34 human samples tested positive for Giardia infection. Among the 34 positive human samples, 55.8% belonged to group A, 14.7% belonged to group B, and 14.7% were a mixture of both group A and group B. A study conducted in Tehran in 2019 successfully identified *Blastocystis* subgroups in 6 out of 72 samples using the HRM method.

**Conclusion**: Early and timely identification of these protozoa is of paramount importance for optimal patient management. The current body of literature indicates that the HRM technique is considered one of the most sensitive methods for detecting medically significant protozoa.

Keywords: HRM, protozoa, intestinal

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Poster

Assessment of parasitic contamination in the soil of public parks Gorgan city

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**Background**: Soil contamination with parasites poses a significant and concerning risk for the transmission of parasitic infections to humans and animals. Recognizing the crucial role of soil as a potential source of parasite transmission, this study aims to investigate the presence of helminth and protozoan parasites in soil of public parks.

**Methods:** In this study, 80 soil samples were collected from 16 public parks in Gorgan city in 2022. The soil samples were collected from various areas within the parks, including flower beds; children's play areas, walking paths, sitting areas, and garbage dumps. To isolate the parasites from the soil, a saturated solution of sodium nitrate was utilized. The samples were carefully examined under a microscope, and all isolates were stained and subjected to further investigation using special acid-fast dyes.

**Results**: *Toxocara* eggs were the most commonly detected parasite, present in 40% of the samples (n = 32). Coccidia oocysts were found in 12.5% of the samples (n = 10), which could potentially be *Sarcocystis* or *Toxoplasma* oocysts. Mites were also detected in 12.5% of the samples (n = 10). Additionally, *Cryptosporidium* oocysts were identified using Ziel-Nelson's modified staining method, and they were found in 10% of the samples (n = 8).

**Conclusion**: Health education plays a vital role in raising awareness about preventive measures.

**Keywords**: Public parks, parasitic diseases, soil contamination

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Assessment of the anti Leishmanial activity of diallyl sulfide combined with meglumine antimoniate on *Leishmania major*: molecular docking, in vitro, and animal model

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**Background**: Currently, no safe vaccine against leishmaniasis is available. So far, different control strategies against numerous reservoir hosts and biological vectors have not been environmentfriendly and feasible. Hence, employing medicinal components along with conventional drugs could be a promising approach to developing novel therapeutic alternatives.

**Methods:** In this study, it is aimed to explore diallyl sulfide (DAS), a dynamic constituent of garlic, alone and in a mixture with meglumine antimoniate (MAT as standard drug) using in vitro and animal model experiments against *Leishmania major* stages. The binding affinity of DAS and four major defense elements of the immune system (iNOS, IFN-y, IL-12, and TNF- $\alpha$ ) was used to predict the predominant binding mode for molecular docking configurations. Herein, we carried out a broad range of experiments to monitor and assess DAS combined with MAT potential treatment outcomes.

**Results**: DAS in combination with MAT displayed no cytotoxicity and employed a powerful anti-leishmanial activity notably against the clinical stage. The mechanism of function involved immunomodulation through the induction of Th1 cytokine phenotypes, triggering a high apoptotic profile and ROS production. This combination demonstrated a significant decrease in cutaneous lesion diameter and parasite load in BALB/c mice. Transmission electron microscopic images illustrated generalized degenerative structural changes at the subcellular level. The histopathological findings performed the infiltration of inflammatory cells associated with T-lymphocytes in alleviating the amastigote stage and improving the pathological changes in BALB/c mice infected with *L. major*.

**Conclusion**: DAS represented potent in vitro and in vivo activities and a more enhanced lethal effect when combined with the standard drug (MAT). Therefore, DAS and MAT deserve further advanced therapeutic development. It should be considered as possible candidates for treating volunteer cases with cutaneous leishmaniasis in designing upcoming clinical trial.

Keywords: Leishmaniasis, diallyl sulfide, combination therapy

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Poster

## Bioinformatics analyses and immunogenic epitopes prediction of ROP17, ROP21, ROP23, ROP25, ROP30 and ROP35 proteins of the *Eimeria tenella*

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**Background**: *Eimeria tenella* (*E. tenella*) is a highly pathogenic and widespread species of *Eimeria* that infects chickens, and it causes a significant disease burden worldwide. Currently, the control of coccidiosis is mainly depended on anticoccidial drugs, but they have many shortcomings.

**Methods:** Different web servers were used to predict antigenicity, allergenicity, solubility, physicochemical properties, post-translational modification sites (PTMs), the presence of signal peptide and transmembrane domains. Besides, the secondary and tertiary structures of the proteins were revealed followed by refinement and validation. At last, NetCTL server was used to predict cytotoxic T lymphocyte (CTL) epitopes, with subsequent immunogenicity analysis. Also, IEDB server was utilized to predict helper T-lymphocyte (HTL) epitopes, followed by IFN- $\gamma$  and IL-4 induction and antigenicity.

**Results**: All assessed proteins were antigenic with the highest antigenicity for ROP21, while ROP17 was not capable of being antigenic. Lack of allergenicity is one of the proper features of a good vaccine candidate. Negative GRAVY score was obtained for all proteins, indicating enhanced interaction with surrounding water-based milieu. Moreover, all proteins possessed high levels of thermostability in a vast range of temperatures, had adequate molecular weight and a durable half-life (30 h). Following secondary structure analysis, random coil was the mostly abundant structure, ranging from 43.81% in ROP30 to 59.18% in ROP21.

**Conclusion**: Totally, these proteins showed appropriate antigenicity, abundant PTMs as well as many CTL, HTL and B-cell epitopes, which could be directed for future vaccination studies in the context of multi-epitope vaccine design.

Keywords: Bioinformatics, ROPs proteins, E. tenella

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Cationized albumin conjugated solid lipid nanoparticles as vectors for delivery of albendazole against cystic echinococcosis

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**Background**: Cystic echinococcosis is a common neglected parasitic disease. Nanoparticles containing drugs have been widely utilized in various formulations for several purposes. The aim of this study was to evaluate the effects of solid lipid nanoparticles containing Albendazole and conjugated to albumin (B-SLN+ABZ) as treatment approach for hydatid cysts in

**Methods:** Albendazole loaded solid lipid nanoparticles was prepared by emulsification and solvent evaporation method. The experimental mice were assessed forprophylactic and therapeutic effects of the drugs. Ultrastructural changes were observed by transmission electron microscopy. A one-way analysis of variance (ANOVA) test was conducted to compare the clinical efficacy study.

**Results**: The variance analysis of the fitted model indicated that the GMS/soy lecithin concentration ratio and the amount of albendazole had a significant effect on nanoparticle size. the GMS/soy lecithin concentration ratio and the amount of albendazole had a notable effect on nanoparticle PdI and EE. During chemoprophylaxis, the B-SLN + ABZ group showed a lower number and weight of cysts ( $0.90 \pm 0.73$  and  $15.01 \pm 10.46$ ) compared to the ABZ + SLN group ( $1.4 \pm 0.51$  and  $26.73 \pm 9.92$ ). Therapeutic efficacy analysis showed a significant reduction in wet weights of metacestodes in mice treated with both B-SLN + ABZ ( $29.37 \pm 13.82$  mg) and SLN + ABZ ( $35.88 \pm 7.49$  mg) compared to the control group ( $59.78 \pm 3.80$  mg).

**Conclusion**: The results showed that B-SLN + ABZ nanoparticles were more effective against E. granulosus cysts compared to free ABZ. The cysts in the animals receiving B-SLN + ABZ every 24 hours showed more ultrastructural changes.

Keywords: Albendazole, albumin, solid lipid nanoparticles Correspondence Email(s): fallah@umsha.ac.ir Poster

Cell and molecular biology in parasitology knowledge: opportunities and challenges ahead

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**Background**: Parasitic diseases cause misery millions of lessfortunate people globally. The knowledge of cellular and molecular biology plays a very important role in the development of parasitology with several opportunities and challenges in the context of cell and molecular biology.

**Methods:** 2. Vaccines: cellular and molecular mechanisms of hostparasite interactions can aid in the development of effective vaccines against parasites. 3. Diagnostic Tools: Genetic mutations or DNA of parasites can be detected through molecular diagnosis 4. Host Immune Response: Studying of host immune responses to parasitic infections provides insights into host defense mechanisms. This knowledge can aid in the development of immunotherapeutic approaches against parasites. 5. Evolutionary Insights: By examining the cellular and molecular changes that occur in parasites over time, researchers can gain insights into the evolutionary dynamics of host-parasite interactions and the factors driving parasite adaptation.

**Results**: Challenges: 1. Complex Life Cycles: Parasites often have complex life cycles involving different stages and hosts. Understanding the cellular and molecular mechanisms underlying these life cycles can be challenging due to the intricate interactions between the parasite and its host(s). 2. Host-Parasite Interactions: Parasites have evolved sophisticated strategies to invade and manipulate host cells. Unraveling the intricate mechanisms underlying host-parasite interactions requires detailed knowledge of the cellular and molecular processes involved. 3. Genetic Diversity: Genetic variations within parasite populations can influence their virulence, drug resistance, and ability to evade host immune responses.

**Conclusion:** 4. Limited Experimental Systems: Some parasitic organisms are challenging to culture or study in the laboratory. This limitation restricts our ability for more experiments and hampers the investigation of cellular and molecular processes in these parasites. The importance of this field of biology in parasitology will be explained in detail.

Keywords: Cell and molecular biology, parasitology Correspondence Email(s): esma@pasteur.ac.ir



Chitosan nanoparticles improve the effectivity of miltefosine against *Acanthamoeba* 

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**Background**: *Acanthamoeba* keratitis (AK) is a corneal sightthreatening infection caused by the free-living amoebae of the genus Acanthamoeba. Early and appropriate treatment significantly impacts visual outcomes. Mucoadhesive polymers such as chitosan are a potential strategy to prolong the residence time and bioavailability of the encapsulated drugs in the cornea.

**Methods:** Chitosan nanoparticles (CNPs) were prepared using the ionic gelation method with negatively charged tripolyphosphate (TPP). The zeta-potential (ZP) and the particle size of MF-CS-NPs were determined. The release profile of MF-CS-NPs was calculated using the dialysis bag method. The cytotoxicity of MF-CS-NPs and MF on the Vero cell line were evaluated at 24 and 48 hours. Also, the hemolytic activity of formulation in vitro and ocular irritation in rabbit eyes were tested.

**Results**: The zeta-potential (ZP) and the particle size of MF-CS-NPs were  $21.8\pm3.2$  mV and  $46.61\pm18.16$  nm, respectively. The release profile of MF-CS-NPs indicated linearity with sustained drug release. The cytotoxicity of MF-CS-NPs on the Vero cell line was 2.67 and 1.64 times lower than free MF at 24 and 48 hours. This formulation exhibits no hemolytic activity in vitro and ocular irritation in rabbit eyes. The IC50 of MF-CS-NPs showed a significant reduction by 2.06 and 1.69-fold in trophozoites at 24 and 48 hours compared to free MF. Also, the MF-CS-NPs IC50 in the cysts form was slightly decreased by 1.26.

**Conclusion:** MF-CS-NPs reduced toxicity and improved the amoebicidal effect of MF. Nano-chitosan could be an ideal carrier that decreases the cytotoxicity of miltefosine. Further analysis in animal settings is needed to evaluate this nano-formulation for clinical ocular drug delivery.

Keywords: *Acanthamoeba*, chitosan nanoparticles, miltefosine Correspondence Email(s): kazemirad@tums.ac.ir Poster

Circulating cell-free DNA in sheep serum with cystic echinococcosis as a diagnosis method

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**Background**: Echinococcosis is a chronic zoonosis caused by larvae of the *Echinococcus granulosus* tapeworms. Imaging techniques are generally used in combination with serological tests. In spite the two methods have limitations. So, in consideration to the limitations, accurate and non-invasive diagnosis this disease is urgently needed.

**Methods:** cfDNA of 35 infected sheep sera were extracted from two different volumes (0.5 and 2 mL) by using the modified phenol-chloroform method, and subjected to both standard PCR and semi nested PCR targeted the mitochondrial gene, NADH dehydrogenase subunit I (Nad1).

**Results**: By standard PCR and semi-nested PCR methods on 0.5 ml of the sera, cfDNA were detected in 8 and 12 out of 35 sheep respectively, while the DNA extracted from 2 ml of each serum, PCR and semi-nested PCR successfully detected cfDNA in 24 and 27 out of 35 sheep sera, respectively. Interestingly, after increasing the template DNA extracted from 2 ml serum, the PCR and semi-nested PCR could detect 29 and 33 out of 35 sheep, respectively.

**Conclusion**: We found that by using a higher volume of sera for DNA extraction, a higher volume of DNA, and using semi-nested PCR method, it is possible to improve the sensitivity of the PCR detection of hydatid cyst cfDNA in sera samples as high as 95%.

Keywords: Echinococcosis, diagnosis, cell-free DNA, PCR Correspondence Email(s): s.h.mirhendi@gmail.com


Comparison of anti-*Toxocara* IgG levels in HTLV-1 positive and negative individuals

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**Background**: Toxocariasis, caused by the parasitic roundworms *Toxocara* spp., can increase anti-*Toxocara* IgG antibodies in infected individuals. HTLV-1 has been associated with various immunological alterations. This study aimed to compare the prevalence of anti-*Toxocara* antibodies in HTLV-1 positive and negative individuals and explore potential factors associated with anti-*Toxocara* positivity.

**Methods:** A total of 171 serum samples were collected, including 78 from HTLV-1 positive individuals and 93 from HTLV-1 negative individuals. Demographic and clinical data, including age, gender, employment status, marital status, dietary habits, and pet exposure, were collected and analyzed for association with anti-*Toxocara* IgG positivity. The presence of anti-*Toxocara* IgG antibodies was determined using ELISA.

**Results**: Among the 171 samples, 3 were positive for anti-*Toxocara* IgG antibodies. Two of these samples were from HTLV-1 positive individuals, and one was from an HTLV-1 negative individual. There was no significant difference between gender, marital status, and occupation. However, all three individuals positive for anti-*Toxocara* IgG antibodies were in the age group of 40-59, and all three resided in urban areas. No significant association was found between the consumption of untreated water and contaminated vegetables, raw or half-cooked meat, contact with animals, pica, and *Toxocara* antibody positivity.

**Conclusion**: Although anti-*Toxocara* IgG prevalence was higher in HTLV-1 positive individuals, non-significant differences were observed. While demographic factors did not show significant associations, the small sample size limits the generalizability of the findings. Understanding these associations is crucial for managing *Toxocara* infections, especially in HTLV-1 co-infected individuals.

Keywords: *Toxocara* infection, HTLV-1, prevalence Correspondence Email(s): salehisgh@mums.ac.ir Poster

Comparison of cytotoxicity of Miltefosine and its niosomal form on chick embryo model

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**Background**: Various drugs have been used for the treatment of leishmaniasis, but they often have adverse effects on the body's organs. In this study, we aimed to explore the effects of one type of drug, Miltefosine (MIL), and its analogue or modifier, liposomal Miltefosine (NMIL), on several fetal organs.

**Methods:** Our in silico approach involved predicting the affinities of MIL and NMIL to critical proteins involved in leishmaniasis, including Vascular Endothelial Growth Factor A (VEGF-A), the Kinase insert domain receptor (KDR1), and apoptotic-regulator proteins (Bcl-2-associate). We then validated and supported these predictions through in vivo investigations, analyzing gene expression and pathological changes in angiogenesis and apoptotic mediators in MIL- and NMIL-treated chicken embryos.

**Results**: The results showed that NMIL had a more effective action towards VEGF-A and KDR1 in *Leishmania*sis, making it a better candidate for potential operative treatment during pregnancy than MIL alone. In vivo, studies also showed that chicken embryos under MIL treatment displayed less vascular mass and more degenerative and apoptotic changes than those treated with NMIL.

**Conclusion**: These results suggest that NMIL could be a better treatment option for leishmaniasis during pregnancy.

Keywords: Leishmaniasis, miltefosine, noisome, cytotoxicity, angiogenesis

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culture medium is not suitable for the transformation of amastigotes into promastigotes, but it is suitable for the cultivation, growth and survival of promastigotes, liken as RPMI1640. Therefore, the new medium is benefitfull and cost effective in comparison to RPMI1640.

Keywords: Leishmania, new culture medium, NNN

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Background: Coccidiosis of domestic chicken is an important disease caused by any of seven species of Eimeria, which, by developing within the epithelial cells of the intestine, cause lesions therein. In order to find the best combination of preserving oocytes in freezing and long-term conditions, the present research was carried out.

Methods: Eimeria spp. oocysts concentrated by centrifugation, and resuspend in SPGA medium (Sucrose: 74.6210 g/litre, KH2P04: 0.5171 g/litre, K2HP04: 1.2541 g/litre, L glutamic acid: 0.9079 g/litre and BSA: 10 g/litre) containing dimethylsulphoxide (DMSO) or 10% glycerol, RPMR-1660 containing 10% glycerol and 10% chicken serum, and chicken serum containing 10% glycerol to a concentration of about 2.5  $\times$ 106 /mL. This mixture dispensed into plastic, screw-capped microtubes and placed in a freezer and leave at -70 °C for at least 5 h and up to overnight. After that, microtubes transferred to liquid nitrogen.

Results: After testing the viability rate of oocysts by the in vivo method and feeding them to the 2-week-old chickens, it was observed that the best result, the highest survival rate, and the ability to cause contamination were observed in the samples stored in the SPGA containing 8% DMSO.

Conclusion: Studies about coccidiosis vaccine depend on the ability to maintenance of sporulated oocysts in the freezing condition, and our research made a comparison about different formulation of preservation conditions.

Keywords: Eimeria, coccidiosis, chicken

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Comparison of Leishmania culture in a new culture medium

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Background: Leishmania is a common zoonotic parasite that causes Leishmaniasis, which has been recognized as a global issue in 98 countries. One of the most reliable methods to diagnose disease is parasite culturing, the most common media used are NNN and RPMI1640 but these media are expensive and difficult

Methods: This study is a laboratory study. To prepare the new culture medium, we used agar, ground semolina, dextrose, blood, gentamicin, distilled water and 33.33% dextrose water serum. NNN medium was made based on standard protocol and RPMI1640 was purchased. After culturing of amastigote and promastigote, the number of parasites counted daily for 28 days



Comparison of molecular and parasitological methods for diagnosis of human trichostrongylosis

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**Background**: Human trichostrongyliasis is a zoonotic disease, which is prevalent among rural populations in some countries. This study was performed to evaluate of various parasitological methods and PCR for diagnosis of human trichostrongyliasis.

**Methods:** A total of 206 fresh stool samples were collected from residents of endemic villages of northern Iran. All samples were examined by conventional parasitological methods including wet mount, formalin ethyl acetate concentration (FEAC), agar plate culture (APC), Harada-Mori culture (HMC) and Willis and along with PCR technique.

**Results**: Among the 206 individuals examined, 73 people (34.8%) were found infected with *Trichostrongylus* species by combined parasitological methods. Considering the combined results of parasitological methods as the diagnostic gold standard, the Willis technique had a sensitivity of 91.7% compared with 52.8% for the APC, 40.3% for the HMC, 37.5% for FEAC and 5.6% for wet mount techniques. Diagnostic specificity was 100% for all the parasitological methods. Moreover, the PCR method detected *Trichostrongylus* spp. DNA in 79 fecal samples (38.3%) with a sensitivity of 97.2% and specificity of 93.3%.

**Conclusion**: According to our findings, the Willis method is more sensitive than other parasitological methods in the diagnosis of human trichostrongyliasis. In general, PCR assay was more sensitive and more reliable in detection of human trichostrongyliasis in comparison with parasitological methods.

**Keywords**: Human trichostrongyliasis, diagnosis, parasitological methodes

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Poster

Comparison of the diagnostic performance of antigen B purified from sheep hydatid cyst fluid (HCF) with commercial ELISA Kit

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**Background**: Cystic echinococcosis (CE) is a zoonotic parasitic disease caused by the metacestode of *Echinococcus granulosus*. CE is a health problem in Middle Eastern countries, such as Iran. The purpose of this study was to purify subunit 8 kDa antigen B from crude sheep hydatid cyst fluid (HCF).

**Methods:** 28 sera samples were collected from hydatid cyst patients who had surgery for a hydatid cyst and had their disease confirmed by pathology after the surgery. Furthermore, 35 samples of healthy individuals with no history of hydatid cysts were collected, as were nine serum samples from parasite-infected non-CE patients. HCF was obtained from sheep fertile cysts at a Sari slaughterhouse and used as an antigen. In an indirect ELISA test, the B antigen was employed, and the results were compared to those from a commercial ELISA kit.

**Results**: The commercial ELISA kit showed 17 cases (23.6%) positive, 44 cases (61.1%) negative, and 11 cases (15.3%) borderline. B antigen showed that 18 (25%), 43 (59.7%), and 11 (15.3%) were positive, negative, and bor-derline, respectively. One sample (1.4% of 72 total samples) of 35 serum samples from healthy individuals was positive using B antigen-based ELISA. In addition, all nine-serum samples from parasite-infected non-CE patients were negative for both tests. The sensitivity and specificity of the commercial ELISA kit have been evaluated at 60.7% and 100%, respectively. For B antigen-based ELISA, these values are 64.3 and 97.7%, respectively.

**Conclusion**: Antigen B produced from hydatid cyst fluid is a promising option for serological identification of hydatid cysts in both infected and healthy individuals. In an indirect ELISA test, hydatid fluid antigen could be used as a precise source of detection.

**Keywords**: Antigen B, cystic echinococcosis, *Echinococcus granulosus*, ELISA

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Comparison of the presence of *Neospora caninum* genome in blood of bovine immunodeficiency virus (BIV) infected and healthy cattle

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**Background**: *Neospora caninum* is a protozoan parasite with global distribution, which mainly causes abortion and reproductive problems in cows. The coexistence of this parasite with factors could suppress the immune system and probably affect its pathogenicity.

**Methods:** The blood samples of 143 cows were collected randomly from the cattle farms of Hamedan city and were analyzed by PCR method for the presence of BIV genome and *Neospora caninum* parasite.

**Results**: 98 out of 143 examined cows were infected with BIV, and the parasite genome was detected in the whole blood of 78 of them. 51 cases of BIV positives (52%) and 27 cases of BIV negatives (60%) had neosporosis (Odds ratio = 0.723, P = 0.375).

**Conclusion**: Based on the recent findings, it seems that there is no significant relationship between these two infections due to the difference in the way they are transmitted, and bovine immunodeficiency virus infection has no effect on the re-activation of neospora latent cysts.

**Keywords**: Cattle, concurrent, *Neospora*, bovine immunodeficiency

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Poster

Comparison of therapeutic effect of Nanonivasha herbal ointment with glucantime on skin lesions caused by *Leishmania major* in BALB/c mouse model

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Medicine, Isfahan University of Medical Sciences, Isfahan, Iran; <sup>4</sup> Department of Parasitology and Mycology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

**Background**: Since, the antibacterial effects of Nano Nivasha herbal cream ingredients (honey, beeswax, vitamins A and E, argan and hazelnut oils) have been confirmed separately, their combination can have an increased anti-leishmanial effect. Therefore, this study investigated the therapeutic effect of Nivasha nano cream compared to glucantim.

**Methods:** 30 Balb/c mice infected with *L.major* were divided into three groups, positive control = 10, test = 10 and negative control = 10. The positive control and test groups received intralesional glucantime and Nanonivasha herbal cream (2 times daily), respectively and the negative control received no treatment.

**Results**: The lesion size median was  $(16.7 \pm 5.6)$  before and  $(9.17 \pm 4.06)$  after treatment in positive control (P < 0.00) and  $(15.7 \pm 6.08)$  before and  $(10.61 \pm 7.6)$  after treatment in test group (P 0.00). Furthermore, the mean period of treatment was 5.4 ( $\pm 1.84$ ) weeks and 4.9 ( $\pm 1.15$ ) weeks in positive control and test groups, respectively (P = 0.34). There was insignificant differences in lesion size after treatment between test and control groups (P = 0.6).

**Conclusion**: Nanonivasha herbal cream can have synergistic effects with glucantime in the treatment of CL.

Keywords: Cutaneous leishmaniasis, glucantime

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Contamination of vector snails with the larval stages of trematodes in selected areas in Northern Iran

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**Background**: Identification of freshwater snails and possible trematodes transmission sites are essential to continue monitoring the potential for disease outbreaks in areas with a history of parasitic infections. We aimed to search some areas in the margin of the Caspian Sea, northern Iran to identify the snail fauna of this area.

**Methods:** More than 5,308 snails from 51 diverse and permanent habitats were studied from April 2019 to October 2021. Snails were collected randomly and identified using shell morphology. Trematode infection in snails were investigated by the release of cercariae and dissection methods.

**Results**: Five families of freshwater snails including Lymnaeidae, Physidae, Planorbidae, Bithyniidae, and Viviparidae were investigated in the Caspian Sea Litoral of Iran. Physidae were found as the most prevalent snails (55.1%) followed by Lymnaeidae (29.4%). The parasitize rate was observed as 20% using releasing cercaria technique. Echinostomatoidea (31%), Schistosomatoidea (8%), and Diplostomoidea (21%), and Plagiorchioidea (40%) were seen as detected parasites. Meanwhile, 60% of the studied snails illustrated the other stages of trematodes.

**Conclusion**: The rate of infection of snails with different cercaria in northern Iran is significant. It needs further deep studies to clarify the situation of zoonoses transmitted by snails in the region. Policy makers should pay attention more to this area in terms of monitoring the snail-transmitted diseases.

**Keywords**: Vector snails, trematode, parasitology, Iran **Correspondence Email(s)**: roknimoh@tums.ac.ir/shahabsarvi@yahoo.com Poster

*Cryptosporidium* molecular investigation and species identification in children with clinical symptoms at northeast of Iran

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**Background**: Cryptosporidiosis is a protozoan disease that can commonly cause fever, nausea, vomiting, stomach pain and diarrhea in children. This research aims to investigate *Cryptosporidium* with molecular method and identification of its species in children with clinical symptoms at northeast of Iran.

**Methods:** In this project, 138 children who had symptoms of acute gastroenteritis and had visited Dr. Sheikh Hospital during the summer of 2020 to 2022. Stool samples were taken and were divided into two parts, one part to perform a direct test and acid-fast staining, the second part was placed in a negative 20 freezer for DNA isolation. Then, PCR method was used to amplify the RNA gene (18S rRNA; SSU-rRNA). Finally, 15 positive PCR products were randomly selected from among the positive ones, and sent to Pishgam Company to determine the genotype and species of the parasite.

**Results**: The average age of people is 44 months (approximately 3.6 years old), the highest age is 144 months (12 years old) and the lowest age is 3 months. Based on direct testing and acid fast staining, 32 patients (23.2%) and based on the test results, PCR, 37 patients (26.8%) were positive for *Cryptosporidium*. Also, the average age of the patients showed that younger people are more infected with Cryptosporidium. Among the 15 PCR products sent for sequencing, 14 samples were diagnosed as *C. parvum* and one sample as *C. hominis*.

**Conclusion**: *Cryptosporidium* infection is high in children with diarrhea in Mashhad city and it is considered as a major health problem in this region. Considering the transmission of this parasite through contaminated water and livestock to human, special attention should be given on children's health, nutritional hygiene especially water.

Keywords: Cryptosporidium, children, gastroenteritis, molecular method Correspondence Email(s): Hoseinifr@mums.ac.ir



Cytotoxicity and Anti-leishmanial activity of new pyrimidopyrimidine derivatives on amastigote of *Leishmania major* 

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**Background**: Pentavalent antimony compounds have been proposed for the treatment of cutaneous leishmaniasis, but due to the reported side effects and resistance, researchers are still looking for new drugs.pyrimidopyrimidine are compounds whose antimicrobial and therapeutic effects have been reported in the past. Accordingly, this study aimed to evaluate the anti-leishmanial effects of 4 pyrimidopyrimidine derivatives.

**Methods:** In this study, concentrations of 9-288  $\mu$ g/ml of each of the pyrimidopyrimidine derivatives (A-D) were prepared by dissolving in DMSO. The cytotoxicity of these concentrations on J774 mouse macrophage cells was investigated for 48 hours by MTT assay. The antileishmanial effect of these derivatives in different concentrations on amastigotes of *Leishmania major* was also investigated in cell culture.

**Results**: The results of the MTT assay showed low toxicity for all derivatives in all concentrations, and cell viability were observed above 50%. Also, the effect of the derivatives on infected macrophages showed IC<sub>50</sub> of 68.4  $\mu$ g/mL, 57.6  $\mu$ g/mL, 67.5  $\mu$ g/mL, 184.3  $\mu$ g/mL for derivatives A, B, C, and D, respectively, which were effective in a dosedependently.

**Conclusion**: The results of this study showed that different pyrimidopyrimidine derivatives studied have low cytotoxicity. Due to their effects on amastigotes of *L. major* can be considered by further studies in the treatment of cutaneous leishmaniasis.

**Keywords**: *Leishmania*, amastigote, pyrimidopyrimidine, cell culture **Correspondence Email(s):** hatam908@yahoo.com Poster

Cytotoxicity of Amphotericin B and AmBisome: In silico and in vivo evaluation employing the chick embryo model

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## <sup>1</sup> Leishmaniasis Research Center, Kerman University of Medical Sciences, Kerman, Iran.

**Background**: Leishmaniasis has been identified as a significant disease in tropical and subtropical regions of the world, with Iran being one of the disease-endemic areas. Various treatments have been applied for this disease, and amphotericin B (Amp B) is the second line of treatment.

**Methods:** In vivo analysis was done by checking pathological changes, angiogenesis, and apoptosis alterations on eggs treated by Amp B and AmBisome. In silico approach was employed to predict the affinity of Amp B and AmBisome to the vascular endothelial growth factor A (VEGF-A), its receptor (KDR1), apoptotic-regulator proteins (Bcl-2-associated X protein (Bax), B-cell lymphoma (Bcl-2), and Caspase-8.

**Results**: The ADME-toxicity prediction reveals that AmBisome possesses a superior pharmacological effect to Amp B. The best result of all the dockings in the Molegro Virtual Docker (MVD) was obtained between Bax, Bcl-2, Caspase-8, KDR1, and VEGF-A targets. Due to the lower Egap (HOMO–LUMO) of AmBisome, the chemical reactivity of AmBisome was higher than that of Amp B. In vivo analysis showed that embryos that received Amp B exhibited less vascular density than AmBisome. Amp B alone significantly increased the expression of apoptosis and decreased angiogenesis genes compared to AmBisome.

**Conclusion**: Overall, the results suggest the potential benefits of AmBisome over Amp B, which might be a better treatment strategy to treat leishmaniasis during pregnancy.

Keywords: Amphotericin B, leishmaniasis, chick embryo

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Deployment of the serum level of egr-miR-2a-3p as a potential

biomarker for diagnosing cystic echinococcosis

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Bahreini<sup>3</sup>, Ali Asghar Dastyar<sup>3</sup>

Poster

Detection of circulatory E. granulosus-derived cell-free DNA in the plasma and urine of human cystic echinococcosis using an in-house PCR: a promising potential diagnostic biomarker

Bentolhoda Habibi 1 @, Shirzad Gholami 2, Mahdi Fakhar 3 ©, Abouzar Bagheri <sup>4</sup>, Mahdi Torabi <sup>5</sup>, Rabeeh Tabaripour <sup>6</sup>, Alimohammad Moradi <sup>7</sup>

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<ol> <li><sup>1</sup> Department of Parasitology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran</li> <li><sup>2</sup> Cellular and Molecular Research Center, Medical Basic Sciences Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran</li> <li><sup>3</sup> Department of Surgery, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran</li> </ol>	<sup>1</sup> Student Research Committee, Mazandaran University Medical Sciences, Sari, Iran; <sup>2</sup> Toxoplasmosis Research Center, Communicable Diseases Institute, Faculty of Medicine, Mazandaran University of Medical Sciences, S Iran; <sup>3</sup> Mazandaran Registry Center for Hydatid Cyst Mazandaran University of Medical Sciences, Sari, Iran; Department of Clinical Biochemistry-Biophysics and Genetics Immunogenetics Research Center Faculty of
<b>Background</b> : Cystic echinococcosis (CE) is a chronic parasitic disease caused by the metacestodes of <i>Echinococcus granulosus</i> senso lato. The larval stages of this parasite, hydatid cyst, are usually diagnosed using imaging modalities and serological testing; however, several studies have recently suggested using the parasite-derived microRNAs (miRNAs) as novel diagnostic biomarkers.	<ul> <li>Medicine, Mazandaran University of Medical Sciences, S.</li> <li>Iran; <sup>5</sup> Baghiatallah Hospital, Baghiatallah University of Medical Sciences, Tehran, Iran; <sup>6</sup> Student Research</li> <li>Committee, Mazandaran University of Medical Sciences, S.</li> <li>Iran; <sup>7</sup> Department of General Surgery Division of HPB a transplantation surgery, Tehran University of Medical Sciences, Sciences, Tehran, Iran</li> </ul>
<b>Methods:</b> The present study included 31 CE patients who were older than 5 years and were admitted to the hospitals of Ahvaz, Iran for hydatid cyst removal surgery during 2019–2021. The egr-miR-125-5p and egr-miR-2a-3p levels were evaluated in the sera of the CE patients before and 6 months after the surgery using Quantitative Real-Time PCR (qRTPCR), and the results were compared with the serum samples from 15 healthy volunteers.	<b>Background</b> : The diagnostic tool for identifying c echinococcosis (CE) patients at an early stage is currently lack However, circulatory cell-free DNA (cfDNA) has shown pote as a biomarker for parasitic infections. It could be used diagnosing CE.
Then, the intergroup comparisons were performed using the t test. <b>Results</b> : patients were between 6–72 years (mean age of 34.6 years). Moreover, one patient (3.2%) had CE1, 14 patients (45.2%) had CE2, 5 patients (16.1%) had CE3, 2 patients (6.5%) had CE4, and 9 patients (29%) had CE5. Also, 21 patients (67.74%) had a positive antigen test using the ELISA method, while 10 patients (32.26%) had a negative ELISA. The pre-operative expression	<b>Methods:</b> The plasma and urine samples were collected from patients with confirmed CE through imaging and histopatholog techniques. All plasma samples were tested for anti-echinoco antibodies using a commercial ELISA test. Total plasma and u cfDNA were extracted and an in-house PCR assay was develo to detect E. granulosus specific cfDNA in the samples of patients.
level of egr-miR-2a-3p was 10.36 folds higher compared to 6 months after the surgery, with an AUC value of 0.8176. However, the expression levels of egr-miR-125-5p did not change significantly 6 months after the surgery compared to pre-operative levels.	<b>Results</b> : Out of the 39 patients, 30 tested positive for E. granul using serology, with a sensitivity of 76.9%. Moreover, detection rates for the cfDNA were 79.5% in plasma samples 58.97% in urine samples using the 80 bp COX1 gene. The plased PCR and serology test showed the highest agreement (K. = 0.53).
<b>Conclusion</b> : According to the present study results, the serum levels of egr-miR-2a-3p can be a promising non-invasive biomarker for diagnosing CE and monitoring its potential recurrence after cystectomy.	<b>Conclusion</b> : Plasma-based PCR has been found to be a reliding diagnostic tool for identifying CE patients at different cyst stat It offers validity, speed, and sufficient sensitivity, making alternative to serology in diagnosing CE in endemic areas.
Keywords: MicroRNAs, <i>Echinococcus granulosus</i> , egr-miR-2a- 3p, egr-miR-125-5p Correspondence Email(s): beiromvandm@gmail.com	Keywords:       Cystic       echinococcosis,       cfDNA,       Echinoco         granulosus,       PCR,       serology       correspondence       Email         mahdifakhar53@gmail.com/sgholami200@gmail.com       Email       correspondence       Email



Detection of helminth eggs in the faeces of wild boar (Sus scrofa) in Lorestan Irovince, Western Iran

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**Background**: Wild boar (*Sus scrofa*) is an ungulate with worldwide distribution. Although it is found in many parts of Iran, there is little data on its helminth infections. In this study, we aimed to investigate the status of helminthic eggs in the feces of *S. scrofa* in Lorestan province, Western Iran.

**Methods:** Overall, 52 samples of wild boar feces were collected during 2021-2023 from Lorestan Province, located on the western edge of Iran. The samples were transferred to the diagnostic helminth laboratory at the School of Public Health, Tehran University of Medical Sciences. The samples were kept at -20 °C until examination by parasitological methods, including formalin-ether concentration and flotation techniques.

**Results**: Among 52 samples from wild boar, eggs of *Trichuris suis* and *Dicrocoelium dendriticum* were detected in 5 (9.6%) and 13 (25%) feces, respectively. Coinfection was found in 4 (7.7%) of samples.

**Conclusion**: The free-ranging activity of *S. scrofa* as reservoirs for many parasites increases their role in the maintenance of parasitic zoonoses. The findings of the current study indicate that roaming of wild boar in habitats close to human settlements has the risk of helminth infections for humans and domestic animals.

Keywords: Helminth eggs, Sus scrofa,

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Detection of *Leishmania* DNA and Blood Meal Identification in Sand Flies in Dezful District, Iran (2018-2019)

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**Background**: Leishmaniasis is transmitted by the bite of infected female phlebotomine sandflies. The sandflies inject the infective stage from their proboscis during blood meals. The aim of present study was an epidemiological investigation on the vector(s) of cutaneous leishmaniasis in rural areas of Dezful city, during April 2018 to January 2019.

**Methods:** Sand fly samples were collected by using sticky traps from indoors and outdoors in different parts of Dezful city during April 2018 to January 2019 and were subjected to molecular methods for detection of leishmanial parasite and blood meal identification by ELISA.

**Results**: Out of the 2132 collected sand flies, *Leishmania* DNA was detected by PCR in 4 females, specifically in one genus identified based on morphological features as *Phlebotomus papatasi*. The ELISA assays revealed that 8 (25%) samples fed on humans. Seven of 8 human blood-fed samples were *P. papatasi* and one seropositive sample in human blood was *P. sergenti*.

**Conclusion**: This is the first molecular report of parasite infection of *P. papatasi* in Dezful City. The results indicated that *P. papatasi* was the primary vector of the disease. Considering the presence of *P. papatasi* as the dominant species in the region which is a component of disease vectors of

Keywords: Leishmania DNA, sand fly, Dezful

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Detection of *Leishmania major* infection in the reservoir host of zoonotic cutaneous leishmaniasis (ZCL) by sequencing of the second internal transcribed spacer(ITS2) of the ribosomal RNA gene in northeast of Iran

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**Background**: In different foci of zoonotic cutaneous leishmaniasis (ZCL) in Iran, *Rhombomys opimus* has been approved as main reservoir host in central and northeast regions. Thus, the current study aimed to identify *Leishmania major* among the reservoir hosts in Shahroud county as one of the ZCL regions in northeast of Iran.

**Methods:** Active colonies of rodents were detected and rodent samples were captured using Sherman live traps. The collected rodents were searched for the presence of any lesions. Two smears obtained from the serous fluid of each lesion were stained by using the Giemsa and then, were investigated under the light microscope. After screening and confirming for amastigote forms, DNA extraction were done from the smears. Finally, the polymerase chain reaction (PCR) was carried out and *Leishmania* species were confirmed after sequencing of PCR products of a fragment of ITS2 and the blast analysis.

**Results**: In the current research, all the captured rodents were morphologically characterized as *R. opimus*. Our findings revealed that of 30 *R. opimus*, six cases were to be infected with *Leishmania* amastigote forms by using the microscopic examination that were positive for *L. major* using the PCR technique. Moreover, *L. major* species were confirmed in *R. opimus* by the sequencing analysis of the PCR products.

**Conclusion**: In the present study, *L. major* infection in *R. opimus* was detected and confirmed for the first time in Shahroud. The findings of this study suggest that the control of these rodents is necessary for the control and prevention of ZCL in this location.

Keywords: Leishmania major, reservoir host, Shahroud Correspondence Email(s): fa57asgarian@gmail.com Poster

Determination and differentiation of western blotting patterns of *Toxoplasma gondii* tachyzoites in seromolecular positive patients

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**Background**: Although *Toxoplasma gondii* rarely causes symptoms in healthy immune system individuals, early-accurate diagnosis using sensitive and specific methods is essential for preventing and treating severe toxoplasmosis in immunodeficiencies. If the acute/chronic phases are correctly and accurately distinguished, the antigen-pattern difference between the two phases can be investigated.

**Methods:** This study collected clinical samples requested to evaluate the antibody titer against *T. gondii* from the medical diagnosis referral laboratories. After re-evaluation with the ELISA, the consistent result samples within the laboratory were evaluated with ELISA-Avidity and Real-time PCR, then grouped in acute and chronic phases to determine the difference in the antigenic pattern by western-blotted.

**Results**: Two bands were observed in the 50-80 kDa of the western blot pattern of all groups. In the western blot pattern of the IgM+/IgG+ group, a 30-35 kDa band was detected. In the IgM-/IgG- serum group, a band was observed only in the 50-80 kDa region. In the only patient of the IgM-/IgG- group with the identification of the RE gene, a band was observed only in 50-80 kDa. In eight of the 12 cases in which the RE gene was detected, in addition to the bands in the 50-80 kDa region, a band in the 30-35 kDa range was also detected.

**Conclusion**: Prescribing the method of evaluation of patients to investigate toxoplasmosis should be accompanied by knowledge of the individual's immune system status. Also, in cases where the anti-parasite antibody titer is not negative, at least two methods should be used to confirm the results.

 Keywords:
 IgG-avidity,
 Real-time
 PCR,
 seroconversion,

 Toxoplasma
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Determining the frequency of low-risk and high-risk genotypes of HPV virus and its effects on related cancers in northeast of Iran

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**Background**: The aim of the study was to determine the frequency of high risk, low risk genotypes and the risk of HPV in the development of cancer associated with suspected infections in the laboratories of Mashhad University of Medical Sciences during 5 years.

**Methods:** HPV infection was diagnosed by PCR and the selected technique for genotyping was Inno-Lipa method. The steps of study included identifying a person suspected of infection with a physician, taking a sample and isolating DNA, performing PCR for diagnosis, and finally performing Inno Lipa Hybridization to determine the type of genotype.

**Results**: 326 patients were studied. The mean standard deviation of patients' was  $32.68 \pm 8.5$  with an age range of 5-66 years. 293 patients were female and 63 patients were male. HPV genotype was identified in 187 patients. The most common genotype was genotype 6 in 35 patients (low risk) and then genotypes 11, 16 (high risk) and 53 identified in 8 cases. Out of 293 females, 148 cases had HPV and the most common genotypes were genotype 6 in 25 patients, genotype 52 and 53 in 7 patients. In the women, the most common low risk was the genotype 6.

**Conclusion**: The results show that prevalence of multiple genotype infection has been increased in comparison with a few years ago. The most common low risk was the genotype 6 while the high risk one was genotype 16 in male against the previous studies was 51.

Keywords: Frequency, HPV, genotype, risk, cancer Correspondence Email(s): Hoseinifr@mums.ac.ir

Poster

Development and evaluation of miltefosine-loaded nanoemulsions on in vitro culture of *Toxoplasma gondii* 

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**Background**: *Toxoplasma gondii* is a common parasite in the world. Pharmaceutical options for toxoplasmosis treatment are limited. Several studies have been conducted on the anti-infectious properties of miltefosine (MLF). We investigated the effectiveness of nanoemulsion miltefosine (NEM) in tachyzoites of *T. gondii*, RH strain.

**Methods:** Various NEM formulations were designed considering pseudo-ternary phase diagrams. Physicochemical properties of developed nanoemulsions (NEs), including pH, polydispersity index (PDI), droplet size, and refractive index (RI) were evaluated. The considered formulation was analyzed for dilution and stability tests. MTT assay was performed on Vero cells for calculation CC50 and on Vero cells with RH strain tachyzoite for calculation IC50. Sulfadiazine (SDZ) and pyrimethamine (PYR) were positive controls. The trypan blue method was used to investigate the effect of drugs (NEM, MLF, SDZ, PYR) in reducing the number of infected Vero cells and reducing the intracellular proliferation of tachyzoites.

**Results**: The final particle size of NEM was calculated to be 17.463 nm by DLS and TEM. The CC50 of NEM (75.7  $\mu$ g/mL) indicated lower toxicity than the other drugs. IC50 obtained by trypan blue, MTT, and test tube methods showed that NEM (28.43  $\mu$ g/mL) has a suitable IC50 against *Toxoplasma* tachyzoites.

**Conclusion:** The calculated selectivity index (SI) demonstrated that NEM (SI = 2.66) is a more suitable drug candidate than the MLF and positive controls. The trypan blue assay indicated the excellent reduction effect of NEM on *T. gondii* intracellular proliferation rate and the number of infected cells.

Keywords: Miltefosine, nanoemulsion, *Toxoplasma gondii*, Toxoplasmosis

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Development and in-house validation of a multiplex-PCR method for detection and identification of *Lophomonas* spp.

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**Background**: Pulmonary lophomoniasis is an emerging disease caused by the protozoan parasite *Lophomonas* spp. Microscopic examination as a routine diagnostic method has limited sensitivity and specificity. Recently, polymerase chain reaction (PCR) methods have been developed and are more accurate.

**Methods:** This study was conducted at the Iranian National Registry Center for Lophomoniasis (INRCL) and included 120 patients clinically suspected of having lophomoniasis, as well as 30 broncho alveolar lavage (BAL) specimens that were confirmed by microscopic examination. The specimens were examined using three methods: microscopic examination (Giemsa staining), conventional PCR, and multiplex-PCR. Moreover, multiplex-PCR was used for simultaneous identification of two species of *Lophomonas*.

**Results**: Among the three techniques, multiplex-PCR was the most sensitive (100%, 95% CI, 85.9–100), while Giemsa staining had the lowest sensitivity (86.7%, 95% CI, 68.35-95.64%). There was good agreement between multiplex-PCR and conventional PCR in identifying positive samples. The study also confirmed the presence of *L. blattarum* species in all samples using by multiplex-PCR.

**Conclusion**: This study demonstrates that in-house multiplex PCR is a sensitive and accurate diagnostic test for the detection and identification of *Lophomonas* species. Therefore, our findings suggest that this method may be a valuable tool to overcome some diagnostic pitfalls for lophomoniasis.

Keywords: Lophomonas, diagnosis, microscopic examination, conventional

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Poster

Development of a new nanoemulgel formulation containing Fluconazole with an effective effect in the treatment of cutaneous *leishmaniasis* 

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**Back ground:** This study aimed to address these challenges by investigating the use of Nanoemulsions (NEs) to enhance the efficacy of FLU against *Leishmania major*(*L.major*).

**Methods:** Nanoemulsions (NEs) containing 0.25% (w/w) Fluconazole (FLU) were prepared using low-energy emulsification at different ratios of surfactant/co surfactant. Several physicochemical characteristics, including particulate size, pH, viscosity, refractive index, and stability, were evaluated. The J774.A.1 cell line was exposed to logarithmic-phase promastigotes, and then amastigotes formed inside macrophages were confirmed by microscopic observation. The effect of free FIU and FLU-NE on *L. major* promastigotes and amastigotes was evaluated in three-time intervals of 24, 48, and 72 hours by MTT assay and microscopic observation. We then investigated the anti-parasitic properties of these optimized FLU-NEs in vivo.

**Results:** The selected formulation of NE contained 2% (w/w) oleic acid as oil, 20% (w/w) Tween 20 as co-surfactant, and 10% (w/w) ethyl alcohol as surfactant. The selected formulation had an average particle size of  $10.51\pm0.24$  nm and a dispersity index of  $0.19\pm0.03$ . The Physicochemical properties of the selected formulation for skin delivery were acceptable, and no instability was observed. The comparison of the effects of free FLU and FLU-NE on different forms of *L. major* showed that the IC<sub>50</sub> of FLU-NE was lower than that of a free form of the drug in all three time intervals.

**Conclusion:** The current results showed that FLU-NE prepared can be used as an acceptable drug delivery system to increase the bioavailability of FLU. So, the current formulation is an efficient drug delivery strategy that, by using a lower concentration of the drug, has a better effect against *L. major*.

Keywords: Nanoemulsion; Fluconazole (FLU); *Leishmania major(L.major)*.

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# Development of nano-carriers in the treatment of *Leishmania*

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**Background**: Leishmaniasis is parasitic diseases in tropical and subtropical regions of the world. It is strongly believed that the development of vaccines is the most appropriate approach to control *Leishmaniasis*. There is no vaccine available yet and the lack of an appropriate adjuvant delivery system is the main reasons.

**Methods:** Leishmaniasis treatment strategies have limitations, and today the approach is towards nanoparticle drug delivery systems such as liposomes. Liposomes are structures of two layers of phospholipid and cholesterol, which include hydrophilic and hydrophobic parts in their structure and provide the possibility of loading hydrophobic, hydrophilic or amphiphilic compounds. Since the *Leishmania* parasite is mainly present in macrophages, drug delivery systems such as liposomes improve the therapeutic index of anti-*Leishmania* drugs and reduce the effective dose along with off-target toxic effects resulting from their non-specific bio-distribution.

**Results**: Targeting of liposomal drug delivery systems is achieved not only by using their different structural features such as particle size and surface charge, but also by attaching different ligands to the surface of liposomes for targeted drug delivery.

**Conclusion**: Nowadays, carbohydrate -coated liposomes, cationic liposomes, arsenoliposomes, peptide-targeted liposomes, Virosome, Niosome and ISCOMs are used to deliver traditional anti-*Leishmania* drugs to *Leishmania*, and due to the importance of drug delivery systems and their many advantages, research in this field has received a lot of attention.

Keywords: Liposome, Virosome, Niosome, ISCOMs, Leishmania Correspondence Email(s): ahmadmehravaran55@gmail.com Poster

Diagnosis of human fasciolosis in serum samples

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**Background**: Fascioliasis is a zoonotic disease caused by a parasitic trematode belonging to the Fasciola genus, and it is known to have a high global prevalence. A total of 70 human samples were analyzed, This study examined the concordance between ELISA and real-time PCR techniques for detecting fascioliasis in serum samples.

**Methods:** A total of 70 human samples were analyzed, comprising definitive positive, suspected positive, and negative specimens, as well as samples from individuals infected with other types of worms (including *Toxocara*, *Strongyloides*, *Taenia*, *Echinococcus granulosus*, and *Trichinella*) and protozoa (such as *Toxoplasma* and *Leishmania*) that tested negative for Fasciola infection. The analysis was conducted using two distinct methods: ELISA and Real-time PCR.

**Results**: In this investigation, it was ascertained that 69.84% (44 out of 63) of the samples tested positive with both methods, and no cross-reactivity with other parasites was detected. Notably, patients who underwent treatment and were referred for a second evaluation were analyzed separately. Of particular interest, the outcomes of the treated group exhibited little correspondence with those of other groups.

**Conclusion**: This study examined the concordance between ELISA and real-time PCR techniques for detecting fascioliasis in serum samples. Our findings demonstrate that both methods are in agreement with respect to the defined objectives and collectively establish that real-time PCR is a viable approach for monitoring patient treatment progress and confirming ELISA.

Keywords: *Fasciola*, serum, ELISA, Real-time PCR Correspondence Email(s): Zibaeim@sums.ac.ir



Diagnostic Accuracy of Intestinal Protozoa Infections in South Khorasan Province Medical Laboratories

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**Background**: Intestinal parasitic infections are considered a health problem in Iran and most third-world countries. Misdiagnosis is a common medical laboratory error and can affect the quality of life and unnecessary use of medications. Errors in medical laboratories are not always life-threatening.

**Methods:** 1800 stool samples were randomly taken from patients in South Khorasan Province who were sent to medical laboratories from April 2020 to March 2021. Among available data on 327 individuals who were admitted to 10 medical laboratories. The stool samples and recorded reports from laboratories were gathered randomly, then were transferred to the Parasitology Research Laboratory of Birjand University of Medical Sciences, Birjand, Iran, and were examined again using routine stool examination (saline and Lugol wet mount) and also complementary methods (formalin ether concentration, acid-fast and tri-chrome staining) by standard protocols.

**Results**: There were differences between our tests'results and medical laboratories' records; these differences were confirmed by applying complementary techniques. The misdiagnoses occurred in 43 (13%) subjects, including 33 (10%) and 10 (3%) as false-negative and false-positive respectively. Usually, most of the cases of misdiagnosis were related to not reporting *Blastocystis* sp. and false positive reporting of *Chilomastix mesnili*. According to the false positive and negative reports, the sensitivity, specificity, and efficiency of the microscopic examination of this study are 65.98%, 95.65%, and 86.85%, respectively, based on the special formula.

**Conclusion**: There was less non-diagnosis or misdiagnosis of pathogenic protozoa in this study. One of the main reasons for misdiagnosis, in addition to insufficient skills, the job dissatisfaction of laboratory personnel and not spending adequate time to examine samples, can be due to the lower quality of the usual laboratory methods

Keywords: Accuracy, intestinal protozoa, misdiagnosis, south Correspondence Email(s): ahaghighi110@yahoo.com

Poster

Different liver hydatid cysts stage based on ultrasonic data in hospitalized patients in Bojnurd

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**Background**: Hydatid cyst is a chronic parasitic worm infection caused by the larval stage of *Echinococcus granulosus* in many parts of the world. The liver is the most common organ involved in this disease. Ultrasound is very effective in diagnosing cysts in the abdominal area, providing important diagnostic information.

**Methods:** This retrospective study reviewed the records of 47 patients who underwent surgery for liver hydatid cysts between 2015 and 2019 at Bojnurd hospitals. Data were collected from hospitalized patients' records, including demographic information, clinical details, and diagnostic ultrasound findings. Cyst prevalence was analyzed based on these data.

**Results**: Among the 47 patients examined, 27 were male (57.44%) and 20 were female (42.56%), ranging in age from 9 to 93 years. Grading results of operated liver cysts revealed that 25 patients (53.19%) had CE1 type cysts, 9 patients (19.14%) had CE2 type cysts, and 4 patients (8.51%) had CE3b type cysts. Additionally, 8 patients (12.02%) had CE5-type cysts, while 1 (2.12%) had a CE4-type cyst. CE5 cysts were more prevalent in older patients. Sixty-three percent of patients had cysts with a diameter greater than 5 cm (medium to large), while only 37% had cysts smaller than 5 cm (small).

**Conclusion**: Ultrasound is a highly suitable method for diagnosing, classifying, and selecting treatment for liver cysts. Expanding the use of ultrasound to detect cysts could improve recovery outcomes for patients with hydatid cysts in different regions.

Keywords: Hydatid cyst, Bojnurd, ultrasound, liver Correspondence Email(s): reza\_shafi@yahoo.com



Different profiles of ARG1 and iNOS gene expression in macrophages of three different rodents exposed to *Leishmania major* and salivary gland homogenates of *Phlebotomus papatasi* 

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**Background**: Early interactions between *Leishmania*-macrophages of host and immunogenic proteins of sand fly saliva, are central to leishmaniasis outcome. We have explored expression of ARG1 and iNOS genes in macrophages of BALB/c, C57BL/6 and *Rhombomys opimus* in presence of *Leishmania major*, SGH of *Phlebotomus papatasi* and *L. major* + SGH.

**Methods:** Stationary phase of promastigotes was used; salivary glands were extracted from female of Ph. papatasi (3 to 5 day-old/non-blood fed). SGH prepared by sonication. Macrophages harvested from the peritoneal cavity of each rodent and grouped as follow; 1) macrophage (control group), 2) MQ + *L. major* 3) MQ + SGH 4) MQ + *L. major* + SGH. After 6 hours of incubation, culture medium supernatant collected, RNA extraction and cDNA synthesis performed, expression level of desired genes checked by Real-time PCR.

**Results**: ARG1 expression pattern in MQ + SGH and MQ + *L. major* group showed the highest and lowest expressions level respectively in BALB/c and C57BL/6. But, in MQ + *L. major* + SGH, although the highest ARG1 expression happened in BALB/c again, the lowest one observed in R. opimus. On the other hand, iNOS expression showed significant increase in all treated group of C57BL/6 macrophages. Interestingly, iNOS expression showed significant differences in MQ + *L. major* group of C57BL/6 in comparison to other rodents.

**Conclusion:** Expression of ARG1 and iNOS in macrophages of three rodents, are different and can justify their clinical outcome of disease. This difference is related to the genetics of host that can affect the immune responses caused by saliva proteins even of the same species of sand fly.

Keywords: ARG1, iNOS, saliva, *Rhombomys opimus*, *Leishmania* major Correspondence Email(s): v.vaziri@sbmu.ac.ir; yasamaryan@gmail.com Poster

Differential gene expression (DGE) analysis in persons with a history of giardiasis

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**Background**: Giardiasis, which is caused by *Giardia duodenalis*, has clinical symptoms such as steatorrhea and can be very dangerous in children. In addition, some documents reported that this parasite is present inside the tissue of patients with cancer.

**Methods:** In this study, we analyzed the gene expression profiles of some main genes important to apoptosis and antiapoptosis in humans. Expression profile arrays of Genomic Spatial Event (GSE) 113666, GSE113667, and GSE113679 obtained from Gene Expression Omnibus were used for meta-analysis using R commands. Cytoscape and STRING databases used the protein–protein Interaction network. Then, the Kyoto Encyclopedia of Genes and Genomes and Gene Ontology analysis was performed. Similar genes in Homo sapiens were identified using Basic Local Alignment Search Tool analysis. The validation was performed on eight people using real-time Polymerase chain reaction.

**Results**: Analysis of the expression of serum amyloid A1 (SAA1), Regenerating Islet-Derived 3 Gamma (REG3G), and REG3A genes did not show any difference between the two groups of healthy and diseased people. Examining the mean expression of the four genes AKT1, CDKN2A, KRAS, and PIK3CA showed that three genes of AKT1, CDKN2A, and KRAS had increased expression in people with a history of giardiasis compared to healthy people.

**Conclusion**: We showed that the gene expression pattern differs in apoptosis and anti-apoptosis signaling in people with a history of giardiasis. *Giardia duodenalis* seems to induce post-non-infectious symptoms with stimulation of human gene expression.

Keywords: Giardia duodenalis, cancer, candidate genes

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#### Poster

Differentiation of cerebral cystic echinococcosis (CCE) from coenurosis using morphometric and molecular methods

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**Background**: Cerebral cystic echinococcosis (CCE) and coenurosis are caused by the larval stages of *Echinococcus granulosus* sensu lato (s.l.) and *Taenia* spp., respectively. Due to the similarity between the symptoms and clinical samples of CCE and cerebral coenurosis, this study aimed to compare CCE and coenurosis using morphometric and molecular methods.

**Methods:** In the present study, four isolates of human cerebral echinococcal cysts, three isolates of *Coenurus cerebralis* from sheep, and one non-cerebral *Coenurus* from sheep were evaluated. Three protoscoleces were separated from each isolate, stained, cleared, and mounted using Formaldehyde Alcohol Azocarmine Lactophenol (FAAL) on a glass slide. Using micrometry, three large and three small hooks from each protoscolex were measured for several morphometric characters. The molecular characterization was carried out using the partial NADH dehydrogenase1 (Nad1) gene. Phylogenetic analysis was performed using the maximum likelihood method.

**Results**: The results showed that in fertile cysts, the total size of the large and small hooks of *Coenurus* was larger than cerebral echinococcal cyst. These parameters demonstrated significant morphological differences between protoscoleces of *C. cerebralis* and cerebral echinococcal cysts. Molecular methods identified the cerebral echinococcal cysts as *E. canadensis* (G6) genotype. One *C. cerebralis* and the non-cerebral *Coenurus* were identified as *Taenia multiceps* and *Taenia multiceps gaigeri*, respectively.

**Conclusion**: This study showed that morphometric indices could be used for differential diagnosis of the fertile cysts of these cestodes. However, in cases with no protoscoleces, molecular methods are essential for the differentiation of CCE from cerebral coenurosis, especially in regions where both diseases are prevalent and endemic.

Keywords: Cerebral cystic echinococcosis, Cerebral coenurosis Correspondence Email(s): smsadjjadi@sums.ac.ir/sadjjadi316@gmail.com Poster

Effect of *Alhagi maurorum* hydroalcoholic extract on preventing secondary hydatid cysts in BALB/c mice

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**Background**: Surgery is the main treatment for hydatid cyst disease, during which the spillage of live protoscoleces is a major cause of hydatidosis recurrence. This study assessed the scolicidal effects of *Alhagi maurorum* hydroalcoholic extract on developing secondary cysts in BALB/c mice infected with *Echinococcus granulosus* parasites.

**Methods:** BALB/c mice were inoculated intraperitoneally with viable protoscoleces. One month after the infection, the mice were randomly divided into 4 groups (7 per group). Group 1 (control group) was orally administered PBS daily. Group 2 was orally administered albendazole daily (150 mg/kg). Group 3 was orally administered *A. maurorum* hydroalcoholic extract daily (500 mg/kg). Group 4 was orally administered *A. maurorum* hydroalcoholic extract daily (250 mg/kg). After 6 weeks of treatment, the weight (g), size (mm), number, and fertility of hydatid cysts were measured after the euthanasia and necropsy of all mice.

**Results**: The weight (g), size (mm), and the number of cysts was significantly lower in the groups receiving 500 mg/kg *A. maurorum* hydroalcoholic extract, 250 mg/kg *A. maurorum* hydroalcoholic extract, and 150 mg/kg albendazole than in the control group (P 0.05). The fertility percentage of hydatid cysts did not show a significant difference between the four groups (P 0.05). Moreover, there were no statistically significant differences in the parasitological parameters between the three groups: *A. maurorum* hydroalcoholic extract 500 mg/kg, *A. maurorum* hydroalcoholic extract 500 mg/kg (P 0.05).

**Conclusion**: The findings demonstrated the effect of *A. maurorum* hydroalcoholic extract against protoscoleces in BALB/c mice. However, cellular and molecular research should be carried out to gain a deeper understanding of the various effects of this extract on hydatid cysts.

Keywords: Alhagi maurorum, BALB/c Mice, hydatid

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Effect of *Ferula assa-foetida* hydroalcoholic extract against flukes of *Dicrocoelium dendriticum*, in vitro

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**Background**: Dicrocoeliasis belong to the group of food borne trematode infections and is one of the most important parasitic zoonosis caused by *Dicrocoelium dendriticum* with geographically widespread distribution. The current study aims to determine the anthelmintic properties of *Ferula assa-foetida* hydroalcoholic extract as an herb in *D. dendriticum* treatment, in vitro.

**Methods:** A plant-based study at 400, 600, 800, and 1000  $\mu$ g/ml for *D. dendriticum* with Closantel (positive control) and RPMI medium (negative control) were prepared. Minimum five worms were separated exposed to the *F. assa-foetida* extract. Each treatment was repeated thrice at the 5% Co2 incubator and the number of lively and dead worms was watched at 0, 12, and 24 hours post-treatment. Mortality time was determined by observing the motility of the flukes and examined under the microscope. The anthelmintic efficacy was measured by scanning electron microscopy technique. The MTT assay is done to evaluate cell viability in culture media.

**Results**: Our experiment by MTT assay on *D. dendriticum* showed, at concentrations of  $1000\mu$ g/mL of hydroalcoholic extract of *F.assa-foetida*, 85.7% of the Hella cells were alive, thus percent of toxicity were 14.3% in 24 hours. According to SEM technique, at a concentration of  $600\mu$ g/mL, no swelling or blisters were observed in the worm treated, but in concentration of  $800 \mu$ g/mL, swelling and blister changes were observed on the parasite's tegument. The mortality rates and the anthelmintic properties of *F. assa-foetida* were highly relied on time and concentration, as far as increasing the time and concentration cause increasing the mortality rate.

**Conclusion**: No previous reports have assessed the effect of F. *assa-foetida* on liver flukes *D. dendriticum*. Therefore, the present study provides a basis for future research on control of these trematodes. It is recommended to document and investigate indigenous knowledge of possible medicinal plants to plan scientific trials that may justify their endorsement.

**Keywords**: *Dicrocoelium dendriticum*; *Ferula assa-foetida*; SEM, MTT

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Poster

Effect of limon extract against *Leishmania major* (MRHO/IR/75/ER) in vitro

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**Background**: Pentavalent antimonials are considered the main treatment of leishmaniasis, but this treatment has multiple side effects. Therefore, it is necessary to replace a suitable and effective treatment against leishmaniasis. In this study, we assessed the effect of lemon extract on the viability of *L. major* (MRHO/IR/75/ER).

**Methods:** The promastigotes of *L. major* (MRHO/IR/75/ER) were cultured in RPMI 1640 medium with 10% fetal calf serum and antibiotics. Each group was prepared with 6x106 promastigote. The promastigotes were exposed with the lemon peel extract with the final concentrations of 2.5, 5, 10, 20, 40, and 50 mM at 25 °C for 72 h. The control groups were the media without parasites and exposure material. After incubation, the viability of the parasites was evaluated using the apoptotic DNA ladder kit as the protocol.

**Results**: Our study showed that the exposure of *Leishmania* promastigotes to different amounts of lemon extract (from 50 to 1000 micrograms) caused the death.

**Conclusion**: Since the has been proven and This study showed that all studied concentrations of lemon extract can cause the death of parasites probably due to antioxidant property of lemon extract. It seems that this substance can be used as a suitable treatment agent against cutaneous leishmaniasis.

**Keywords**: *Leishmania major*, treatment, lemon, cutaneous

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Effect of Rosuvastatin-loaded chitosan nanoparticles in the intracellular tachyzoites of *Toxoplasma gondii* 

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**Background**: New therapeutic methods are needed for treatment of acute toxoplasmosis. Statins, especially Rosuvastatin, a class of lipid-lowering medications, can reduce *Toxoplasma gondii* replication. This study aimed to evaluate the effect of Rosuvastatin loaded - chitosan nanoparticles in the intracellular tachyzoites of *T. gondii*.

**Methods:** In this study, Rosuvastatin loaded chitosan nanoparticle (CH-NP-ROS) was synthesized. Then, size distribution (DLS) of CH-NP-ROS and Surface charge (ZP) was determined and Giemsa staining by different concentrations of Rosuvastatin (ROS), and Rosuvastatin loaded chitosan nanoparticle (CH-NP-ROS), was performed to evaluate the number of tachyzoites on murine Macrophage cells (MQ).

**Results**: In this experiment, Rosuvastatin - Loaded Chitosan Nanoparticles (CH-NP-ROS) were synthesized. Surface charge (ZP) and size distribution (DLS) of CH-NP-ROS were determined to be +32.5 mv and 233.87 nm. The results demonstrated that CH-NP-ROS decreased the number of tachyzoites, compared to ROS. Statistical analysis showed that CH-NP-ROS significantly killed 91%, (P 0.05) of *T. gondii* tachyzoites compared to ROS 84%.

**Conclusion**: Nanoformulation is a promising approach for increasing bioavailability of free Rosuvastatin and is safe for using therapeutic effects in *Toxoplasma gondii*.

**Keywords**: *Toxoplasma gondii*, tachyzoite, rosuvastatinloaded-chitosan-nanoparticles Rosuvastatin

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## Poster

Effect of testosterone and progesterone on propagation of *Toxoplasma gondii* in in vitro

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**Background**: Previous studies revealed alterations of sex hormones; especially testosterone, in infected humans and rodents, but little is known about the effects of sex hormones on the propagation of *T. gondii.* 

**Methods:** The glioblastoma cells (U-87MG) were treated with different concentrations of testosterone and progesterone and the infection was done by tachyzoites of the RH strain of *T. gondii*. The number of infected cells, viability of *T. gondii*-infected cells, and parasite burden were measured by direct counting under a light microscope, MTT assay, and quantitative real-time PCR (qPCR), respectively.

**Results**: The results showed that testosterone at concentrations of 100 and 250 nM significantly increased the number of infected cells and parasite burden 24 and 48 hours post treatment compared to untreated controls. Progesterone had no significant effects in the same manner.

**Conclusion**: The results indicated that testosterone could augment the propagation of *T. gondii* in in vitro, but progesterone had no significant effects in the same manner.

Keywords: T. gondii, hormone, androgen, estrogen

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Effect of three transgenic *Leishmania* species expressing fusion gene of mLLO-Bax-Smac in the apoptosis of the infected macrophages

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**Background**: So far, no confirmed vaccine for *Leishmanias* is infection has been reported. Generating transgenic parasites to express proapoptotic proteins like BAX through the parasite itself accelerate the infected macrophage apoptosis and prevent *Leishmania* differentiation. Hence, we examined the effect of transgenic *L. infantum, L. major, L. tropica* expressing mLLO-BAX-SMAC proteins in accelerating macrophage apoptosis.

**Methods:** Materials and Methods: The entire coding sequence mLLO-Bax-Smac was designed and cloned into the pLexyNeo2 plasmid. The designed sequence was integrated downstream of the 18srRNA locus into the mentioned *Leishmania* spp. genome using homologous recombination. PCR and Western blot techniques, and hemolysis test were used to confirm integration accuracy and evaluating mLLO-BAX-SMAC fusion protein expression. Next, the transgenic parasites pathogenesis was investigated compared with wild-type *Leishmania* spp. in vitro and in vivo.

**Results**: Results: The results of PCR and Western blot confirmed proper integration and expression of mLLO-Bax-Smac fusion protein in three *Leishmania* spp. The flow cytometry results revealed faster apoptosis of infected macrophages by three transgenic *Leishmania* spp. compared with wild-type parasites-infected macrophages. Moreover, less virulent as a mild lesion with the less parasitic burden of the spleen and liver was observed only in infected mice by transgenic *L. major* and *L. infantum*.

**Conclusion**: Conclusion: The data suggested that transgenic *L. major*, *L. infantum*, and *L. tropica* expressing mLLO-BAX-SMAC construct could be used as an experimental model for developing vaccination against *Leishmanias*is.

Keywords: *Leishmania*sis, Apoptosis, Transgenic, Homologous Correspondence Email(s): Maryam.aghaei2008@gmail.com Poster

Effectiveness of miltefosine nanoemulsion in the treatment of acute and chronic toxoplasmosis

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**Background**: *Toxoplasma gondii* is one of the most common parasites worldwide. It is of great importance to identify new potential drugs effective and less harmful in pregnant women and newborns. We investigated nanoemulsion miltefosine (NEM) in the treatment of acute and chronic toxoplasmosis.

**Methods:** A combination of triacetin, Tween 80, and ethanol (1:2) was used for the development of NEM formulations. To investigate the performance of miltefosine (MLF), NEM, sulfadiazine (SDZ), and pyrimethamine (PYR) (positive control) in vivo, acute toxoplasmosis was induced in mice by an intraperitoneal injection of RH strain tachyzoites. After five days, the mice were examined for the number and condition of tachyzoites and histopathological changes in the liver and spleen. Chronic toxoplasmosis was investigated in rats and the number and size of brain cysts along with histopathological changes were assessed in different groups.

**Results**: The results of the in vivo assessment of drugs in acute toxoplasmosis showed the following order regarding a decrease in the number of tachyzoites and an increase in survival rate: SDZ&PYR NEM MLF. The effects of drugs on chronic toxoplasmosis showed a significant effect of NEM (50%) on reducing the number of cysts compared to SDZ&PYR (10%) and MLF (12%) and reducing the size of NEM brain cysts (21%) compared to SDZ&PYR (5%) and MLF (8%).

**Conclusion:** Increasing the penetration of NEM through the bloodbrain barrier (BBB) and subsequently reducing the number and size of *T. gondii* tissue cysts is a promising new drug in the treatment of chronic toxoplasmosis.

Keywords:	Foxoplasmosis,	miltefosin	e, nanoemulsion,
Toxoplasma goi	ıdii		
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#### Poster

## Effects of silver nanoparticles coated apigenin on *Trichomonas vaginalis*

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**Background**: *Trichomonas vaginalis* is causing trichomoniasis, the most common sexually transmitted infection today. Metronidazole is the major treatment; however, it has side effects, including mutagenicity, carcinogenicity, and it reported cases of resistance. In order to use of nanoparticles coated with herbal compounds can be considered properties and low cytotoxicity.

**Methods:** In the current study, silver nanoparticle coated apigenin was made along with the control drug (metronidazole) with different concentrations was investigated during 24 and 48 hours at 37 °C, that plates was kept in an incubator. The final number of parasites was determined using trypan blue vital staining and neobar slide. In addition, the value of Cytotoxicity Concentration (CC50) was determined via probit regression analysis in SPSS20.

**Results**: The obtained results showed that the nanoparticles are spherical and have an average size of 20 nm. The CC50 values for nanoparticles at 24 and 48 hours were 111.211  $\mu$ g/mL and 81.331  $\mu$ g/mL and The CC50 values for Metronidazole at 24 and 48 hours were 107.09  $\mu$ g/mL and 76.54  $\mu$ g/mL respectively.

**Conclusion**: According to our findings and the observed effects of nanoparticles on *T. vaginalis* trophozoites, these nanoparticles exhibit promise as potential safe drug candidates for trichomoniasis treatment, either alone or in combination with standard metronidazole. However, further validation through in vivo or animal experiments and human studies is warranted

Keywords: Silver nanoparticles, apigenin, *Trichomonas vaginalis* 

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Poster

Emergence of pulmonary lophomoniasis among suspected patients with tuberculosis by a small subunit ribosomal RNA PCR in Darab district, southwestern Iran

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**Background**: Lophomoniasis is a chronic protozoan lung disease with main clinical symptoms such as chronic cough and productive sputum. Due to the high similarity of clinical symptoms between lophomoniasis and tuberculosis (TB), we attempted to determine *Lophomonas* infection among suspected patients with tuberculosis in Darab district, southwestern Iran.

**Methods:** This descriptive-cross-sectional study was conducted on 203 sputum samples of people suspected of tuberculosis who were referred to the tuberculosis laboratory of Darab Health Center, southwestern Iran, during 2021-2022. Demographic and epidemiological information of the patients was recorded. In the morphological method, Giemsa staining was used for *Lophomonas*. The small subunit ribosomal RNA (SSU rRNA) PCR was also used to accurately confirm the presence of parasites.

**Results**: In this study, 203 people suspected of tuberculosis were included, of which 47 samples (23.2%) were positive for *Lophomonas* parasite using PCR method, 2 people (4.3%) lived in urban areas (Darab) and 45 people (95.7%) lived in rural areas. 42 (89.4%) of them were man and 5 (10.6%) of them were woman. The lowest age group of infected with *Lophomonas* was 20 years and the highest age group was 21-40 (55.3%). The most common clinical signs were also cough (83%). There was no coinfection among people with *Lophomonas* infection and tuberculosis (P = 0.58) and only one person had tuberculosis.

**Conclusion**: Our results indicate a relatively high prevalence (23.2%) of *Lophomonas* infection in people suspected of TB in Darab district. Therefore, lophomoniasis should be given high consideration in the differential diagnosis of TB.

Keywords: Darab, lophomoniasis, morphology, PCR, tuberculosis Correspondence Email(s): mahdifakhar53@gmail.com



Emerging of cutaneous leishmaniasis by *Leishmania major* in Mahallat, Markazi province, Iran

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**Background**: Cutaneous leishmaniasis is a disease mainly caused by *Leishmania major* and *L. tropica* in the old world. Recently, we had some reports regarding emerging of the cutaneous leishmaniasis in Mahallat, Markazi province, Iran. In this study, we identified the agents of the cutaneous leishmaniasis emerging in Mahallat using ITS1-PCR-RFLP.

**Methods:** Samples were isolated from the suspicious patients with cutaneous leishmaniasis referred to Health Center of Mahallat, Markazi province, Iran in 2022. The questionnaire and consent informs were completed by patients. The samples were analyzed by light microscopy after Giemsa staining. After observation of *Leishmania* amastigotes, DNA extraction was done using the tissue and blood extraction kit. ITS1-PCR-RFLP was performed in order to detection and identification of the clinical isolates.

**Results**: Our investigation showed that five samples detected *Leishmania* spp. amastigotes using microscopic observation. ITS1-PCR-RFLP identified that all of the clinical isolates were *L. major*.

**Conclusion**: This study showed that the condition of Mahallat, Markazi province, Iran is suitable for emerging cutaneous leishmaniasis and therefore it is necessary to design the prevention and control programs in this region.

**Keywords**: *Leishmania major*, cutaneous leishmaniasis, ITS1-PCR-RFLP

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Poster

Enhancement of Apoptosis in Caco-2, Hep-G2 and HT29 cancer cell lines Following Exposure to *Toxoplasma gondii* Peptides

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 <sup>2</sup> Department of parasitology and mycology, faculty of medical sciences, Tabriz University of Medical Sciences

**Background**: Cancer or neoplasm consequences more than 20 million new cases and 10 million deaths every year. Parasites like *Toxoplasma gondii*, by its components, could modulate the cancer system by inducing apoptosis. The objective of this investigation is to assess the anticancer potential of *T. gondii* derived peptides on cancer cell lines.

**Methods:** Candidate peptide by its similarity to anticancer compounds was predicted through the computer-based analysis/platform. The impact of the peptide on cell viability, cell proliferation, and gene expression was evaluated through the utilization of MTT, flow cytometry, and real-time PCR methodologies.

**Results**: The cell viability rate was significantly decreased (P = 0.001) in all cell lines with a concentration of  $\le 160 \ \mu g$ . During the 48 hours, the IC50 concentrations for HT29 and HepG-2 were found to be the lowest and highest, respectively, measuring 107.2 and 140.6  $\mu g/mL$ . Notably, a significant reduction in the expression levels of Bcl2 and APAF1 genes was observed in the Hep G2 and HT29 cell lines.

**Conclusion**: These findings indicate that the *T. gondii* peptide (TgP) had an impact on cancer cell mortality and caused alterations in the expression of genes related to apoptosis.

Keywords: Neoplasm, *Toxoplasma gondii*, anticancer, peptide

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*Enterobius Vermicularis* eggs in urine: a case report of vulvovaginitis in a 2-year-old girl

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**Background**: Vulvovaginitis is a common condition in pediatric patients, characterized by symptoms such as itching, irritation, redness, and vaginal rash. We present a case of a 2-year-old girl referred to the laboratory for a urinalysis test, ultimately leading to the unexpected discovery of Enterobius vermicularis eggs in the urine sample.

**Methods:** The patient's medical history, clinical symptoms, and urinalysis results were obtained from the referring physician. The urinalysis was performed using standard laboratory techniques, including microscopic examination for the presence of parasites.

**Results**: The 2-year-old girl presented with itching, irritation, and a burning sensation in the vulva, redness, swelling, and a vaginal rash. Urinalysis revealed the unexpected finding of *Enterobius vermicularis* eggs. The presence of the eggs in urine sample was indicative of a parasitic infection, highlighting the necessity for comprehensive clinical assessment.

**Conclusion**: The unexpected discovery of *Enterobius vermicularis* eggs in the urine of a pediatric patient with vulvovaginal symptoms underscores the importance of performing a thorough physical examination. Physicians should be mindful of the potential for parasitic infections in patients presenting with vulvovaginitis, as early recognition and appropriate treatment are crucial for

**Keywords**: Vulvovaginitis, *Enterobius vermicularis*, urinalysis

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Poster

Environmental health factors and cutaneous leishmaniasis (CL) in Northeastern Iran

Hakim azizi <sup>1</sup> © 🕑

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**Background**: Cutaneous leishmaniasis (CL) is one of the main causes of vector-born disease in younger people. To evaluate the association of environmental health factors on the odds of CL incidence, a case-control study was conducted in northeastern Iran.

**Methods:** This study was conducted within 2020-2021 based on individual and household data from a tertiary referral center. Cases were patients diagnosed with CL by PCR method; controls were selected among the patients' relatives, and information was obtained from a health registry system. Demographic and socioeconomic data of 1871 subjects, included age, sex, household information and environmental health factors. Multivariable models with environmental factors in various condition and CL were separately fit by univariate and mixed multiple unconditional logistic regression.

**Results**: Results revealed that the use of well water sources compared to surface water is significantly associated with CL (odds ratio [OR] = 0.204; 95% CI, 0.13-0.33; P = 0.001). Muddy houses, ruined buildings or wastelands and stagnant water, canals and rivers near the houses were also associated with CL (OR = 3.85; 95% CI, 1.66-8.89; P = .002; OR = 2.47; 95% CI, 1.76-3.47; P = 0.001). Besides, the existence of pine tree was found to be a risk factor (OR = 3.25; 95% CI, 2.12-4.99; P = 0.001) and the same goes for the use of waste collection system (OR = 4.43; 95% CI, 3.32-7.51; P = 0.001).

**Conclusion**: Environmental factors related to houses were significantly associated with CL and may represent the modifiable risk factors of CL disease.

Keywords: Leishmaniasis, environmental factors

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5th International & 12th National Congress of Parasitology and Parasitic Diseases of Iran		
NICOPR	Alborz University of Medical, Sciences, Karaj, Iran May 21 - 23, 2024	
Poster	Poster	
Epidemiological aspects of <i>Lophomonas blattarum</i> infection in Mazandaran Province: A national registry-based study	Epidemiological status of <i>Toxoplasma gondii</i> infection in Mazandaran province: A registry-based study	
Rabeeh Tabaripour <sup>1</sup> D, Mahdi Fakhar <sup>2</sup> O, Ali Sharifpour <sup>3</sup> , Mostafa Soleymani <sup>2</sup> , Zahra Hosseininejad <sup>2</sup> , Maryam Nakhaei <sup>2</sup>	Rabeeh Tabaripour <sup>1</sup> D, Mahdi Fakhar <sup>2</sup> O, Zakaria Zakariaei <sup>3</sup> , Zahra Hosseininejad <sup>2</sup>	
<ol> <li><sup>1</sup> Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran</li> <li><sup>2</sup> Iranian National Registry Center for Lophomoniasis, Imam Khomeini Hospital, Mazandaran University of Medical Sciences, Sari, Iran</li> <li><sup>3</sup> Pulmonary and Critical Care Division, Imam Khomeini Hospital, Mazandaran University of Medical Sciences, Sari, Iran</li> </ol>	<ol> <li><sup>1</sup> Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran</li> <li><sup>2</sup> Iranian National Registry Center for Lophomoniasis, Imam Khomeini Hospital, Mazandaran University of Medical Sciences, Sari, Iran</li> <li><sup>3</sup> Toxicology and Forensic Medicine Division, Mazandaran Registry Center for Opioids Poisoning, Imam Khomeini Hospital, Mazandaran University of Medical Sciences, Sari, Iran</li> </ol>	
<b>Background</b> : <i>Lophomonas</i> infection is a respiratory disease in humans that is associated with symptoms of cough, sputum, dyspnea, and sometimes hemoptysis. The present study was aimed to investigate the prevalence of <i>Lophomonas</i> spp., infection in patients who were referred to the Iranian National Registry Center for Lophomoniasis (INRCL).	<b>Background</b> : <i>Toxoplasma gondii</i> ( <i>T. gondii</i> ) is one of the most common parasitic infections in humans and other vertebrates worldwide. This baseline study aimed to investigate serological evidence of <i>Toxoplasma</i> infection among patients who were referred to the Iranian National Registry Center for Toxoplasmosis (INRCT).	
<b>Methods:</b> We examined patients enrolled in the INRCL from 2022 to February 2024 at the Mazandaran University of Medical Sciences, northern Iran. 112 bronchoalveolar lavage fluid (BALF), 43 sputum, 5 pleural tap and 14 Tracheal secretions of the patients were examined by using microscopic examination (fresh and Giemsa-stained smears). To confirm positive microscopic smears, small-subunit ribosomal RNA (SSU rRNA) PCR method was used. <b>Results:</b> Out of 174 specimens, 37 (21.26%) were infected with <i>Lophomonas</i> using microscopic examination including 35 BALF and 2 sputum Among them 18 (48 65%) were inhabitante in urban	<b>Methods:</b> In this retrospective (descriptive-analytical) study, all patients referred to the INRCT, constitute the study population (underlying diseases/status). All data including demographic and some related characteristics were collected into a questionnaire and registered at the Iranian National Registry Center for Toxoplasmosis (INRCT) at the Mazandaran University of Medical Sciences, northern Iran. The existence of anti- <i>Toxoplasma</i> antibodies (IgG) was explored using a commercially available enzyme-linked immune sorbent assay (ELISA) kit (PishtazTeb, Iran), based on the manufacturer's protocol.	
areas and the rest of them in rural areas. The most patients in urban areas and the rest of them in rural areas. The most patients were significantly female. The most clinical finding of the patients was cough. Moreover, the BALF specimen was the most positive clinical sample in diagnose of lophomoniasis. All positive microscopic smears were confirmed by PCR test.	<b>Results</b> : Anti- <i>Toxoplasma</i> IgG were detected among 862/1691 patients (50.98%). The most positive anti- <i>Toxoplasma</i> antibodies were in infertile women and the least of that were in suicide. IgG seropositivity was more common in females (66.01%) than in males (33.99%) and in 31–40 years old patients (33.41%) in urban populations (53.71%).	
<b>Conclusion</b> : Our results showed the high morbidity of lophomoniasis in Mazandaran province, thus the lophomoniasis could be considered as an endemic status in the area.	<b>Conclusion</b> : Our results showed the high prevalence of <i>Toxoplasma</i> infection in infertile women, thus investigation in this group helps to control, prevent and treat it.	
<b>Keywords</b> : INRCL, lophomoniasis, microscopic examination, PCR	Keywords: IgG, INRCT, Mazandaran, Toxoplasma gondii	
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		Alborz University of Medical, Sciences, Karaj, Iran	
			May 21 - 23, 2024

Poster

Essential oil components and antitrichomonal effects of *Piper* nigrum L.

Ali jamshidizad <sup>1</sup> ©, Mohammad Matini <sup>1</sup>, Mohammad Fallah <sup>1</sup>, Dara Dastan <sup>2</sup>, Farid Azizi-Jalilian <sup>3</sup>

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**Background**: Trichomoniasis, caused by *Trichomonas vaginalis* protozoan, is the most common nonviral sexually transmitted infection worldwide. Although metronidazole and tinidazole are the only approved drugs for treatment, drug-resistant cases of infection are on the rise. The aim of this study was the evaluation of antitrichomonal potential of *Piper nigrum* and limonene.

**Methods:** The parasites were treated in vitro with essential oil and different extracts of *P. nigrum* seed and limonene using microtiter plate method. The oil of *P. nigrum* was also analyzed by gas chromatography-mass spectrometry and gas chromatography-flame ionization detector. Furthermore, the cytotoxicity assay of them were screened on Vero cell line by MTTmethod.

**Results**: The tested *P. nigrum* fractions were able to kill 100% of *Trichomonas* trophozoites at minimum lethal concentration (MLC) and reduce the trophozoite viability at sub-MLC and lower concentrations. After 48 hours exposure, the most potent fraction was the n-hexane extract with MLC of 78 µg/mL followed by the essential oil and methanol extract with MLC of 156 µg/ mL, limonene (MLC=1250 µg/mL), and then, aqueous extract with MLC value of 25 mg/mL. Moreover, according to cytotoxicity assay, *P. nigrum* oil was less toxic to Vero cell than limonene, with a selectivity index (SI) of 13.2 and 2.04, respectively.

**Conclusion**: This study clearly demonstrated the trichomonacidal potential of *P. nigrum*. Thus, *P. nigrum* fractions can be considered promising antiprotozoal agents and the basis for further development to discover new phytochemicals compounds.

Keywords: Essential oil, limonene, Trichomonas vaginalis

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Evaluating the expression profile of four antimoan-resistant genes in promastigote form in sensitive and resistant clinical isolates of urban-type cutaneous leishmaniasis in Kerman province

Razieh Tavakoli Oliaee<sup>1</sup> © P, Iraj Sharifi<sup>2</sup>, Ali Afgar<sup>3</sup>

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 <sup>3</sup> Research Center for Hydatid Disease in Iran, Kerman University of Medical Sciences, Kerman, Iran

**Background**: Clinical resistance to pentavalent antimonials has been considered one of the main reasons for treatment failure in anthroponotic cutaneous leishmaniasis (ACL). The main aim of the present study was to determine the expression level of four major resistance markers in resistant and sensitive clinical isolates of ACL in Kerman province.

**Methods:** 30 samples from resistant and sensitive cutaneous leishmaniasis patients were taken from the Kerman Health Center and cultured in the medium of NNN and RPMI-1640 in at 25 °C. To assess the expression of resistance genes, RNA extraction was performed using the Qiagen kit according to its instructions. CDNA synthesis performed using the Prime Script RT reagent Kit based on 100 ng concentration in the Analytical Jena PCR device. Finally, the expressions of ACR2 (Arsenate reductase 2), PRP1 (Pentamidine resistance protein 1), MST (Mercaptopyruvate sulfurtransferase) and HSP70 (Heat-shock protein 70) was measured by real-time PCR using SYBR\_Premix Ex TaqTM II.

**Results**: Gene expression analysis showed the up-regulation of all four genes of ACR2, PRP1, MST and HSP70 by 2.3, 1.5, 3.2 and 2.5 fold, respectively, in resistant compared to sensitive *Leishmania* tropica patients. Also, the isolates represented a strong positive linear correlation between gene expression of HSP70 and MST. A mild positive correlation was observed between HSP70 and ACR2 in isolated samples.

**Conclusion**: In cases of inconclusive outcomes of resistance tests in clinical isolates, expression analysis of a set of influential genes can be beneficial to identify distinctive biomarkers between antimony-resistant and sensitive parasites.

Keywords: Antimonial resistance, Leishmania tropica, markers

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Evaluation of appendicitis caused by *Enterobius* vermicularis in pathology samples of the last 10 years in Bushehr, Iran

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**Background**: The ectopic presence of helminths and their eggs in the appendiceal duct can directly and indirectly with antigenic stimuli lead to the hyperplasia of lymphatic follicles in the wall of the duct and thereby cause duct obstruction and subsequent acute appendicitis.

**Methods:** A number of 3210 slides related to appendix samples of appendectomy patients due to enterobiasis during the last 10 years were collected in Bushehr hospitals. They were analyzed using diagnostic key and microanatomy technique. The results of microscopic evaluation as well as the information in the files of the samples were summarized and the data were analyzed with SPSS statistical software and *chi-square* test.

**Results**: The prevalence of cases of appendicitis caused by *E. vermicularis* in the studied samples was 0.8%. Statistical tests showed that there was a significant relationship between appendicitis caused by *E. vermicularis* and the gender and age of the patients, but there was no logical relationship with the place of residence of the patients.

**Conclusion**: The prevalence of appendicitis caused by *E. vermicularis* has decreased in recent years in Bushehr, which can be attributed to the effect of the covid pandemic. It is suggested that initial evaluations be done for early detection of this parasitic infection, especially in infected areas.

Keywords: Appendicitis, *Enterobius vermicularis*, Bushehr, Iran Correspondence Email(s): afshin914@gmail.com Poster

Evaluation of astacin and HSP70 genes in the larvae and adult stages of *Strongyloides stercoralis* 

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**Background**: Understanding the genetic features of *S. stercoralis* is substantial biological interest, and is essential for identifying new drug targets, diagnostic methods and appropriate prevention. In this study, we evaluated the expression levels of heat shock protein 70 (Sst-hsp-70) and astacin (Sst-ast) in the larvae and adult stages of S. stercoralis.

**Methods:** After culturing the *Strongyloides stercoralis* that was isolated from a hyperinfection patient on the nutrient agar medium, different stages of parasite were collected. The RNA was extracted and cDNA synthesized. The expression levels of astacin and HSP70 genes were evaluated by real-time PCR technique.

**Results**: The higher expression level of Sst-ast (P = 0.032) and Sst-hsp-70 (P = 0.029) genes were observed in L3 larvae compared with adult stages.

**Conclusion**: HSP70 and astacin genes may play an important role in pathogenesis of *S. stercoralis*. By evaluating these genes and potential application of knockdown and knock-in techniques, we can make significant progress in the pathogenesis and treatment of this parasite.

Keywords: Strongyloides stercoralis, larvae, adult, HSP70

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Evaluation of experimental transmission of toxoplasmosis through lactation in BALB/c mice

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**Background**: Due to the importance of transmission of infectious diseases through breast milk and also the lack of sufficient information about parasites, especially *Toxoplasma* parasites, whose presence in breast milk has been proven in studies, the present study aims to investigate the experimental transmission of toxoplasmosis through breastfeeding.

**Methods:** In this study, three groups were considered, each group consisted of three mothers and five newborns. The first group, 3 mothers' mice were injected intraperitoneally with 12-10 *Toxoplasma* PRU strain cysts on day 0 of the offspring's birth. In the second group, mothers were injected with the cysts on the 5th day of lactation, and the third group was injected on the 10th day of lactation. In the fourth, fifth and sixth groups, 50,000 tachyzoites of RH strain of *Toxoplasma* were injected as above on days zero, five and ten of lactation, respectively.

**Results**: 8 weeks after the injection of PRU strain into mother mice, the brains of offspring mice in different groups were examined for the presence of cysts, the possibility of transmission of PRU strain to offspring on days 0, 5, 10 of lactation was 6.6%, 20% and 66.6%, respectively, also in the RH strain after recording the time of death of offspring mice, the probability of transmission was 33.3%, 53.3 and 86.6%, respectively.

**Conclusion**: In the present study, the possibility of transmission of *Toxoplasma gondii* through lactation to infants in mice was confirmed. This is important factor that require further studies.

Keywords: Toxoplasma, lactation, transmission

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#### Poster

Evaluation of induced immune response with DOTAP liposomes containing Imiquimod adjuvant in murine model

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**Background**: Leishmaniasis is caused by intracellular parasites. Adjuvants are the utmost important part of a vaccine, to induce the immune response in the right direction. It seems that the use of a suitable adjuvant and a delivery system is effective in inducing immune reactions for protection against leishmaniasis.

**Methods:** In the present study, it was investigated whether DOTAP liposomes having SLA and imiquimod adjuvant, can induce a Th1 response and protect against *Leishmania major* challenge in BALB/c mice. Studies were performed with Female 6–8 week old BALB/c mice. Assessment of lesion development, type of generated immune reaction and parasite burden in the foot and spleen after challenge with *L. major*.

**Results**: The results displayed that there was considerable difference (P 0.05) between the size of the lesion in group of mice immunized with Lip+ Imiquimod + SLA in comparison with the group of mice immunized with other formulations. The results of parasite burden in spleen and footpad load also confirmed this issue. The highest level of IgG2a and IFN-  $\gamma$  secretion were observed with Lip DOTAP + imiquimod + SLA more than the control (*P* = 0.001).

**Conclusion**: The results of this study show that liposome DOTAP + SLA + imiquimod formulation generates a cellular immune response that is protective against challenge against *L. major*.

**Keywords**: Cationic liposome, *Leishmania major*, adjuvant

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Evaluation of LAMP and multiplex/nested PCR for malaria diagnosis in Zahedan

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 <sup>2</sup> Infectious Diseases and Tropical Medicine Research Center, Zahedan University of Medical Sciences, Zahedan, Iran

**Background**: Malaria is one of the most serious health problems in many countries, including Iran. Accurate diagnosis is important regardless of the elimination status of a country. A cross-sectional study was performed on 105 people who were suspected to be positive for malaria infection in Sistan and Baluchistan, Iran.

**Methods:** DNA was extracted from the prepared thin and thick films for molecular methods. Multiplex/nested polymerase chain reaction (mn-PCR), loop-mediated isothermal amplification (LAMP), and light microscopy (LM) were compared with nested PCR (nPCR) as a gold standard.

**Results:** Of 105 subjects, 52 (49.5%), 58 (55.2%), 58 (55.2%), and 63 (60%) were positive for malaria by LM, nPCR, mn-PCR, and LAMP, respectively. The sensitivity, specificity, and kappa were 92.1%, 100%, and 0.9 for LAMP and 100%, 100%, and 1 for mn-PCR, respectively. Eight cases of coinfection (*Plasmodium vivax* and *Plasmodium falciparum*) that were not detected by LM method were diagnosed by mn-PCR and LAMP.

**Conclusion**: n the present study, the high sensitivity and specificity of LAMP and mn-PCR indicate that these two tests are good alternatives to nPCR for malaria diagnosis

Keywords: Multiplex/nested PCR, LAMP, malaria

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Poster

Evaluation of patients with nasopharyngeal myiasis and cutaneous myiasis in Zahedan, Iran

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 <sup>2</sup> Infectious Diseases and Tropical Medicine Research Center, Research Institute of Cellular and Molecular Sciences in Infectious Diseases, Zahedan University of Medical Sciences,

Zahedan, Iran

**Background**: Myiasis is caused by the presence of fly larvae (maggot) in body tissues. The larvae usually enter through wounds and skin lesions or through natural cavities in the body such as the mouth, ears, eyes, and genitourinary tract. Nasopharyngeal myiasis involves infections of the nose, mouth, sinuses, and ears.

**Methods:** The present study set to identify and determine the species of myiasis-causing flies in patients referred to Imam Ali Hospital in Zahedan, Iran. To identify abundant species of myiasis-causing flies in people referred to Imam Ali Hospital in Zahedan, in terms of developing different kinds of myiasis from 2021 to 2023, 19 larvae samples were collected from five patients and were then examined and identified. Morphological traits such as anterior and posterior respiratory holes and oral sections were used to identify the larvae. The larvae then became adult flies by keeping them in proper conditions after the larval and pupa.

**Results**: Patients referring with acute symptoms had a mean age of 52 years and were all male. All patients were infected in the suburb areas or outside the city. Analysis of the population composition in terms of abundance of myiasis-causing species revealed that *Calliphora vicinia* species from the Calliphoridae family with 47% infection was the cause of nasopharyngeal (Ear) and cutaneous myiasis, and *Lucilia sericata* with 32% infection was the cause of nasopharyngeal (Nose) and cutaneous myiasis. In addition, *Sarcophaga africa* species from the Sarcophagidae family was collected and identified at a rate of 21% as the cause of cutaneous myiasis.

**Conclusion**: Myiasis is an ectoparasites infection of humans and vertebrates. Therefore, due to the risk of damage to vital organs of the body and the possibility of infection, physicians should ask patients to refer to entomology laboratories for further examination and diagnosis of fly species when they show clinical signs.

Keywords: Myiasis, nasopharyngeal, Zahedan, Iran

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NICOPR	Alborz University of Medical, Sciences, Karaj, Iran	
	May 21 - 23, 2024	
Poster	Poster	
Evaluation of relative frequency of <i>Blastocystis</i> subtypes in healthy people and gastrointestinal symptoms in Isfahan	Evaluation of <i>Strongyloides stercoralis</i> risk factors among patients referred to therapeutic centers of Babol	
Zahra Ghayour Najafabadi <sup>1</sup> ©, Somayeh Mousavi Mobarakeh <sup>1</sup> @	Soude Darvishi Ganji <sup>1</sup> Ø, Maryam Ghasemnezhad Jamee <sup>2</sup> , Arshia Yahyazadeh Jelodar <sup>3</sup> ©, Farzane Jafarian <sup>2</sup>	
<ul> <li><sup>1</sup> Department of Medical Parasitology and Mycology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.</li> <li>Background: <i>Blastocystis</i> is an extracellular and immobilized protozoan that is currently one the most common gastrointestinal tract protozoan in humans and a wide range of Animals. <i>Blastocystig</i></li> </ul>	<ol> <li><sup>1</sup> Department of Laboratory Sciences, 17 Shahrivar Hospital, Babol University of Medical Sciences, Babol, Iran</li> <li><sup>2</sup> Clinical Research Development Unit of Rouhani Hospital, Babol University of Medical Sciences, Babol, Iran</li> <li><sup>3</sup> Department of Medicine, School of Medicine, Islamic Azad University, Sari Branch, Sari, Iran</li> </ol>	
is divided into 17 subtypes (STs) based on the small ribosomal subunit gene.	<b>Background</b> : <i>Strongyloides stercoralis</i> is one of the neglected helminthic infections and about 30-100 million people are infected around the world. Immunocompromised patients are more	
<b>Methods:</b> Fecal samples were collected from 160 asymptomatic patients and 328 individuals with gastrointestinal symptoms. The specimens were examined microscopically and all were cultured in <i>Blastocystis</i> specific medium. DNA was extracted from positive	vulnerable to this infection. This parasitic infection can be asymptomatic in immunocompetent individuals, but it is potent to be fetal in patient with underlying disease.	
cultures and then PCR was performed. 69 PCR products were purified and sent for sequencing. To achieve a fast and inexpensive method to differentiate the subtypes, a pair of common primers were designed, then the Real-Time method was performed and their melting curves were evaluated. The results were analyzed by SPSS software.	<b>Methods:</b> The cross-sectional study was conducted between April 2022 to March 2023 among 43 patients that <i>S. stercoralis</i> was detected in their stool samples The <i>S. stercoralis</i> larva in the stool samples were investigated by microscopic examination, and direct techniques such as saline wet mounts and concentration technique were applied.	
<b>Results</b> : Up to 160 (asymptomatic) 33 cases and from 328 (gastrointestinal symptoms) 40 cases were positive for <i>Blastocystis</i> PCR. From the sequenced samples, 4 (ST1), 41 (ST2), 22 (ST3), and 1 (ST7) were obtained. There was no significant difference between the subtypes in the two groups. However, there was a significant difference ( $P = 0.04$ ) in ST2 and ST3 between the two groups. Also, based on the melting curve in the Real-Time method,	<b>Results</b> : Overall, 55.81% of patients were belonged to rural societies and 44.18% of them were from urban area. Among 43 patients that their stool exam was positive for <i>S. stercoralis</i> larva, 6 of them (13.95%) were diabetic patients, 8 patient (18.60%) were diagnosed with cancer, 3 patients (6.97%) were detected with heart failure and 2 patients (4.65%) suffered from pneumonia.	
the Blastocysts were classified into three groups, for ST1 and ST2 the temperature was 81-83, and respectively for ST3 and ST7 74 and 79 $^\circ C.$	<b>Conclusion:</b> S. stercoralis prevalence is depends on its geographical distribution and rural communities are more susceptible. In addition, underlying disorders make patients more unparable to S. stercoralis and diabatic patients are highly	
<b>Conclusion</b> : <i>Blastocystis</i> infection was higher in the asymptomatic group than in the gastrointestinal group. The frequency of subtype 3 in asymptomatic individuals was higher than those with gastrointestinal symptoms. Due to the widespread prevalence of	endangered. Therefore, it is important to detect different risk factors related to strongyloidiasis to prevent mortality in immunocompromised patients.	
Blastocystis, ignoring it can cause many problems for sensitive people in the community.	Keywords: Immunocompromised, parasite, risk factor	
Keywords: Blastocystis, culture media, DNA sequencing	Correspondence Email(s): yahyazadeharshia2@gmail.com	
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Evaluation of the anti-leishmanial effects of the niosomal form of tioxolone, benzoxonium chloride, and their combination on *Leishmania major* in in silico and in vivo assays

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**Background**: Chemotherapy is the mainstay of treatment for leishmaniasis. The gold standard treatment for cutaneous leishmaniasis (CL) is mainly intralesional or parenteral administration of antimonials. Lately, a great deal of research has been done on the development of topical treatment based on single agent or combination therapy.

**Methods:** Following previous reviews about niosomal forms of tioxolone (NT), benzoxonium chloride (NB), and their combination (NTB), in the present study, we used Molegro Virtual Docker (MVD), PyMOL, and Discovery Studio software to study molecular interactions between IL-10, IL-12, and 5-Metacaspase receptors and niosomal drugs. We predicted the 3D structure of 5-Metacaspase using homology modeling by SWISS-MODEL. We identified potential therapeutic alternatives for treating *Leishmania* using in silico ADME (Absorption, Distribution, Metabolism, Excretion) and drug-likeness profiling. Also, an in vivo assay of selected drugs, carried out in BALB/c mice topically and their antileishmanial effects were measured by evaluation of lesion size and splenic parasite burden.

**Results**: NTB has shown potential as a drug for leishmaniasis based on satisfactory results from molecular docking, ADME investigations, and drug-likeness properties. Also, the NTB significantly reduced the size of footpad swelling and the number of parasites in the spleen of mice in comparison with other groups (P < 0.05).

**Conclusion**: Since there were statistically significant differences between the effects of NT and NB with NTB in in vivo tests, and also due to the superior performance of NTB in the in vitro and in silico tests this component should be used for further human efficacy trials, and in-depth mechanistic action.

Keywords: Niosome, Tioxolone, Benzoxonium chloride

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Poster

Evaluation of the biodiversity, distribution and tracking of Leishmania major in sand flies in Harand and Egieh, Isfahan province, central Iran

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**Background**: Isfahan province is one of Iran's cutaneous leishmaniasis (CL) foci, showing a high incidence of the disease every year. Using parasitological and molecular methods, this survey evaluated the fauna, geographical distribution and parasitic contamination of the sandflies in the Jolgeh area of Isfahan province.

**Methods:** The sandflies were collected in May-December 2019 using a sticky trap and sandfly species identification was done by diagnostic key. A kDNA semi-nested PCR was used to detect *Leishmania major* contamination.

**Results**: In total 408 specimens of 1260 collected sandflies were explored, among which 353 and 55 samples belonged to Harand and Egieh, respectively. About 271 samples (66.4%) were female and 33 samples (32.6%) were male. The sandflies belonged to six genera and the highest prevalence belonged to *Phlebotomus papatasi* (212, 52%), *Ph. caucasicus* (165, 40.4%), *Sergentomyia sintoni* (20, 4.9%) and *Ph. kazeruni* (3, 0.7%). The highest indoor and outdoor frequency was for *Ph. papatasi*. Among 180 mosquitoes in molecular assay, only 14 specimens including 9 *Ph. papatasi* and 5 *Ph. caucasicus* were infected with the parasite, with 13 specimens from Harand.

**Conclusion**: In both regions, the vector and the parasite species and their abundance are related to the appearance of wounds in early autumn. Also, all the sandflies infected with the *Leishmania* parasite were caught outdoors. This shows the importance of monitoring the nests of rodents to control and fight.

Keywords: Cutaneous leishmaniasis, sand fly, semi-nested-PCR

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Evaluation of the therapeutic effect of amphotericin B-Sambucus ebulus topical ointment on cutaneous lesions of *Leishmania major* in BALB/ c model.

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**Background**: Leishmaniasis is a zoonotic disease caused by the *Leishmania* parasite. Since no suitable and safe drugs are available, therefore the need for new treatment strategies is urgent. So, this study evaluated anti-leishmanial effect of combination therapy with amphotericin B-*S. ebulus* ointment compared to amphotericin B, invitro and invivo.

**Methods:** The *S. ebulus* was extracted by maceration method. In in vitro study, the concentrations of 100, 200 and 400 µg/mL were used against *L. major* promastigote to evaluate the inhibitory effect by colorimetric assay. Thirty Balb/c mice infected with *L. major* were divided into three groups (Positive control (n = 10), Negative control (n = 10) and test groups (n = 10). After the occurrence of lesion in mice, treatments were daily initiated using S. ebulus topical ointment plus amphotericin B. The size of lesions was measured in day1, 7, 14, 21 and 28.

**Results**: The anti-leishmanial effect of all concentrations was increased during different times and the highest rate was observed at the concentration of 100 mM after 48 h in vitro. The mean of lesions size (mm) significantly decreased in the group treated with the combination therapy compared to the control groups, during time (P < 0.05) and the highest reduction in wound size was related to the concentration of 100 mM.

**Conclusion**: The results of current study indicated that *S. ebulus* topical ointment can have synergistic effects with amphotericin B in the treatment of CL and this combination might be an alternative candidate for current treatments.

Keywords: Leishmania major, Sambucus ebulus

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Poster

Evaluation of vitamin D effects on the growth of Hydatid Cyst

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**Background**: Cystic echinococcosis is a parasitic infection that causes hygiene problems and economic losses. The disease can be treated using surgery and chemotherapy. However, the side effects of current drugs require the development of new ones. Vitamin D regulates innate and adaptive immune responses and is anti-cancer, antimicrobial, and anti-parasitic.

**Methods:** To perform this study, protoscoleces were collected from naturally sheep liver infected with hydatid cysts. Briefly, 1500 protoscoleces with more than 90% viability were injected intraperitoneally into 60 female Balbc mice at least 6-8 weeks old and the same weight. The animals were divided into six equal groups, and vitamin D drop in different doses (0.5, 1, 2, 4, and 8  $\mu$ g/kg/body weight) was orally given one other day, one week before infection for four months. Then blood sample was collected from the heart, and a necropsy was done. All the organs were evaluated for the cysts' number, size, and ...

**Results**: The results showed that low doses of vitamin D could reduce the number and weight of the cysts in infected mice compared to the control group. Significant differences have been shown between the treated and control groups in the length and width of the smallest cyst.

**Conclusion**: The body needs more micronutrients when fighting parasitic infections because many parasites deplete the host's micronutrient levels. In a study, the effect of vitamin D on *Candida albicans* was investigated in mice, and found out the dual effect of this vitamin on the host's immune system response against *Candida*.

Keywords: Vitamin D, hydatid cyst, protoscoleces

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Exploring knowledge, attitudes, perceptions, and willingness to information about keeping dogs and cats and contracting *Toxocara* infection among faculty members and students in Afghanistan

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**Background**: No studies on toxocariasis and/or *Toxocara* infection have been conducted in Afghanistan. However, none has assessed community awareness, knowledge, and practices related to pet contact and toxocariasis.

**Methods:** A community-based cross-sectional study was conducted between October and December 2023. The design consisted of a questionnaire-based survey conducted in the schools as well as the Cheragh Educational Hospital of Ghalib University, Kabul, Afghanistan.

**Results:** A total of 347 participants, comprising 271 (78.6%) males and 74 (21.4%) females with a median age of 25.6 years (range 21 to 30 years), were interviewed. Awareness of the signs and symptoms of human toxocariasis, including transmission and prevention measures, was very low. While the majority had heard about parasitic diseases transmitted by pets, they were not able to link contact with dogs and cats to toxocariasis. Most participants were aware of parasitic helminths in dogs and cats, including their predilection sites, but were not aware of modes of transmission or prevention measures.

**Conclusion**: The study shows overall poor knowledge, attitudes, and practices related to toxocariasis among the community of faculty members and students at Ghalib University. This poses a serious challenge for the control and elimination of *Toxocara* species infections, and thus efforts to improve knowledge, attitudes, and practices should be improved.

**Keywords**: *Toxocara*, awareness, medical academics, Afghanistan

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Poster

Exploring of Acanthamoeba and Lophomonas in the sputum specimens of suspected tuberculosis patients in Babolsar, Mazandaran Province

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**Background**: *Lophomonas* is a newly emerging pathogen, while *Acanthamoeba* is a free-living amoeba. The respiratory tract can serve as a suitable environment for the spread of different species of these two parasites to the lungs. In this study, we aimed to determine the presence of these two parasites simultaneously in sputum.

**Methods:** This descriptive-cross-sectional study was conducted on 201 sputum samples of people suspected of tuberculosis (TB) who were referred to the tuberculosis laboratory of Babolsar Health Center in Mazandaran province, throughout 202-2023. In the morphological method, direct smear and Giemsa staining was used for *Lophomonas* and non-nutrient agar medium for culture of *Acanthamoeba*. Moreover, two conventional PCR methods were used to accurately confirm the presence of both parasites.

**Results**: In this study, 201 people suspected of TB were included, of which 80 subjects (39.8 %) lived in urban areas and 121 (60.2 %) lived in rural areas. Among them, 113 (56.2%) were female and 88 (43.8%) were male. Overall, 23 samples (11.4%) were positive for *Lophomonas* parasite using PCR method, which among them 7 (30.4%) lived in urban areas and 16 (69.5%) lived in rural areas. However, there was not found evidence of *Lophomonas* in the sputum samples using direct smear and Giemsa staining methods. Moreover, there was not found evidence of *Acanthamoeba* in the sputum samples.

**Conclusion**: The present study indicates that, due to the similarity of the clinical manifestations of lophomoniasis and TB, it is important to consider lophomoniasis in the differential diagnosis of TB in the studied areas to prevent misdiagnosis.

**Keywords**: *Acanthamoeba*, *Lophomonas*, sputum, morphology, PCR

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Exploring the role of *Crithidia* spp. and *Leishmania* parasites in cutaneous leishmaniasis: a molecular study in Golestan province, north of Iran

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Background: Cutaneous leishmaniasis is a parasitic infection that important health problem. This study aimed to conduct a molecular investigation of Crithidia spp. and Leishmania parasites in wounds of patients with suspected leishmaniasis in Golestan province north of Iran.

Methods: This cross-sectional descriptive study included 140 patients with suspected cutaneous Leishmaniasis referred to leishmaniasis diagnostic laboratory in Aggala Health Center, in Golestan province, Iran between 2019 and 2020. Therefore, a questionnaire was designed for collecting of demographic and epidemiological data of patients. Samples were collected to create smears from wounds of patients, and they were stained of Giemsa. DNA extracted from smears and PCR was performed for diagnosis of Crithidia spp. and Leishmania.

**Results**: Out of 140 patients, 117 were positive for *L.major*, 3 were positive for L.tropica, and 20 were negative for both parasites. One patient smear was positive for Crithidia species. The analysis of our results mentioned that the Crithidia spp. positive sample had no significant relation with the variables of gender, age, address, ethnicity, occupation, history of CL, type of wounds, number of wounds, and wound location (P > 0.05). There was no significant relation between the frequency distribution of Leishmania and other variables (gender, age group, address occupation, number of wounds, location of wounds) (P >0.05).

**Conclusion**: The epidemiologic aspects and drug resistance of cutaneous leishmaniasis and Crithidia in endemic areas can be used to plan future control and treatment protocols.

Keywords: Cutaneous leishmaniasis, Crithidia, Leishmania, PCR

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Expression of matrix metalloproteinases in human cystic echinococcosis

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Background: Cystic echinococcosis is a zoonotic disease caused by the Echinococcus granulosus senso lato. Parasitederived products have been shown to regulate host matrix metalloproteinases (MMPs), contributing to CE pathogenesis and progressive liver fibrosis in intermediate hosts. The current study aimed to investigate the potential role of MMP1, 7, 8, and 13.

Methods: Thirty CE patients with active, transitional, or inactive hydatid cysts were enrolled in this study to determine the inductive effects of E. granulosus on the expression of MMP-1, MMP-7, MMP-8, and MMP-13 in healthy liver tissue and fibrotic liver tissue using qRT-PCR.

Results: According to the WHO-IWGE classification, patients with functional cysts (CE1 and CE2) had the highest percentage (46.6%). MMP-1, MMP-7, MMP-8, and MMP-13 expression levels were significantly higher in fibrotic liver than in normal liver tissue. MMP-13 and MMP-1 had the highest and lowest expression levels among MMPs. Compared to the normal group, the fold change for MMP-13 in the fibrotic group was greater than 12 and had the highest AUC value (AUC = 0.8283).

Conclusion: Our findings suggest that E. granulosus-derived products might be involved in regulating host MMPs. Thus, MMPs may be considered potential biomarkers for predicting CE prognosis. Because of the non-normal distribution of our patients' CE types, further research, particularly on circulation MMPs, is needed to confirm the potential role of MMPs.

Keywords: MMP-1, MMP-7, MMP-8, MMP-13, CE

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First report of *Enderleinellus krochinae* Blagoveshtchensky, 1965 (Insecta: Anoplura) and Neohaematopinus syriacus Ferris, 1923 (Insecta: Anoplura) in a Persian squirrel Sciurus anomalus Güldenstädt, 1785 (Rodentia: Sciuridae) from Hamedan, Iran

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**Background**: *Sciurus anomalus* is a rodent commonly called the Persian squirrel (also Caucasian squirrel). It is a medium-sized tree squirrel native to temperate broadleaf and mixed forests in the Irano-Anatolian region of western Asia. Here we report mixed louse infestation in a Persian squirrel from western Iran.

**Methods:** On 5th December 2023 a freshly dead adult male squirrel was found in Hamedan city suburb and transferred to the Laboratory of Parasitology. Death was possibly because of rodenticide toxicity. During examinations, tiny moving creatures were observed between body hairs. They were diagnosed to be sucking lice. The lice were collected, cleared in KOH, mounted on glass slides with Canada Balsam, and identified according to the original descriptions or keys.

**Results**: Results: 33 lice specimens were collected and diagnosed as Enderleinellus krochinae Blagoveshtchensky, 1965 (n = 27; 6  $\Diamond$ , 21  $\bigcirc$ ) and *Neohaematopinus syriacus* Ferris, 1923 (n = 6; 2  $\Diamond$ , 4  $\bigcirc$ ).

**Conclusion**: This is the first report of *E. krochinae* and *N.* syriacus from the Persian squirrel in Iran. Considering wide distribution of this micromammal in the Zagros Mountains and recent invasion to the southern slopes of the Alborz Mountains co-distribution of lice species identified herein needs to be elucidated.

Keywords: Sucking louse, squirrel, record, ectoparasite

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Poster

First report on natural *Leishmania* infection of *Phlebotomus papatasi* due *Leishmania major* by high resolution melting curve method in Sabzevar, northeast of Iran

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**Background**: Leishmaniasis is an infectious disease that occurs in cutaneous, visceral or mucocutaneous forms in many parts of the world. The representative of this disease is a single-celled parasite of the genus *Leishmania*, which is often transmitted through sand. Since the development of effective control strategies against the disease requires accurate.

**Methods:** Within the context of a cross-sectional investigation, sandflies were procured from various regions of Davarzan city from April 1401 to September 1401 using sticky traps. Then, the collected samples were mounted and identified using valid keys to determine the species. The species identification was performed using the molecular HRM (high-resolution melting analysis) method.

**Results**: In this particular study, 210 sandfly samples were obtained and carefully analyzed. The results showed that there were 130 sandflies in the animal area and the prevalence was 69.1%. On the other hand, 80 sandflies were collected from human activities and the probability is 38.1%. Of the 118 female midges, 99 were identified as Papaya midges with a prevalence of 42.5%; It was determined that 19 samples were sand with a probability of 8.2%. The most common species in the Darvarzan region is *Phlebotomus papatasi*. More importantly, species identification was performed using the molecular HRM (high-resolution melting).

**Conclusion**: This study represents the inaugural implementation of HRM-Assay for the identification, diagnosis, and determination of the *Leishmania* disease vector within sand flies in the northeastern region of Iran. This new method demonstrates versatility in providing rapid diagnosis of *Leishmania*sis, simultaneous identification of various diseases, and even quantitative assessment of *Leishmania*.

**Keywords**: HRM, Assay, Leishmania, Phlebotomus papatasi, Phlebotomus sergenti

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First survey of epidemiology and associated risk factors of Human intestinal parasitic infections among Gonabad residents, northeast of Iran

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**Background**: Assessing intestinal parasites' prevalence and distribution patterns is essential for designing an efficient control program to enhance public health quality. The present study was conducted to investigate the prevalence of parasitic infections in the human population and identify the related risk factors of Gonabad city, southeast of Khorasan Razavi province.

**Methods:** In this cross-sectional study, 1038 stool samples were examined based on the parasitological method like direct wet-mounting, formalin-ether concentration and the Gomori's trichrome staining. Moreover, the socio-demographic data of inhabitants were recorded.

**Results**: The overall intestinal parasitic infection prevalence was 18.1 %, whereas this value for protozoan agents was 16.7% (95% CI: 14.14 to 19) and 1.3% (95% CI: 0.7 to 2) for helminth parasites. The highest parasitic agents prevalence belongs to Blastocystis sp (12.4 %), Entamoeba coli (1.8%) and Enterobius vermicularis (1.2%),respectively. Giardia lamblia (1.1%)Entamoeba histolytica/dispar (0.3%), Iodamoeba butschlii (0.3%), Chilomastix mesnili (0.3%), Hymenolepis nana (0.2 %) Dientamoeba fragilis (0.2%) and Trichomonas hominis (0.3%) were other identified parasites. Multivariate logistic regression analysis revealed a significant association of parasitic intestinal infections with factors such as age, residency status and source

**Conclusion**: Our findings approved that the protozoan agents have a relatively high prevalence among the studied population. So it could be severe enough to alarm the entities implementing healthcare services measures for parasitic intestinal infection control.

Keywords: Intestinal parasites, helminth, Prevalence, Iran,

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Poster

Free-living predatory Parasitidae mites (Arachnida: Mesotigmata) on calves in a dairy farm

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**Background**: Mesostigmata is a diverse taxon with various types of lifecycles from predatory to free livings. The adults reside in temporary habitats, while the deutonymph stage disperse phoretically on insects. Mesostigmata, including Parasitidae typically prey on the eggs and larvae of Diptera, specifically muscid flies and other small invertebrates found in Iran.

Methods: Its case reoprt.

**Results**: The study documents the annual occurrence of a significant population of Parasitidae mites on young calves at a dairy farm. Over two consecutive years in the beginning of spring, calves younger than two months old were heavily infested with a high number of mites. This infestation coincided with the calves experiencing varying levels of anemia. The mites were collected and sent for specific diagnosis as the anemia was thought to be related to hematophagous acari e.g. *Dermanyssus* spp.

**Conclusion**: he mesostigmatid mites were found to be empty of blood and the plate's tand chaetotaxy identified them to differ from parasitic Mesotigmata. The mites were identified as free-living parasitid mites though their presence on the body of the animals isnt common and have not been reported in the reviewed literature

Keywords: Parasitidae, mites, *Dermanyssus* spp.

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Frequency detection of *Trichomonas vaginalis* of men referred to Fertility and Infertility Research Center Hamadan University of Medical Sciences, Hamadan, Iran, 2023

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**Background**: This study is aimed to detect the frequency of trichomoniasis, a sexually transmitted infection caused by an anaerobic protozoan *Trichomonas vaginalis*, in men referred to the Fertility and Infertility Research Center Hamadan University of Medical Sciences.

**Methods:** In this cross-sectional study, a group of 197 male volunteers who sought medical attention for issues related to infertility were participated. The urine and semen samples were collected in the sterile conditions. Both urine and semen sediment were promptly examined under a microscopy to detect the presence of motile trophozoites. Subsequently, 50 microliters of urine sediment were inoculated into the Dorse culture medium, whereas 50 microliters of semen sediment were inoculated at 35.5oC. Finally, the processed urine samples were kept for molecular analysis.

**Results**: The investigated subjects had an average age ranging from 36 to 40 years. There were 181 volunteers with fertility issues, outnumbering the 16 individuals with normal fertility. Spermogram analysis showed that 48% of participants had non-motile or progressive sperms, and 48% had abnormalities in sperm morphology. *T. vaginalis* was not detected through microscopic assessment, but PCR and sequence analysis revealed one case in a 33-year-old infertile individual, who had only 0.3% normal sperm with 19% motility.

**Conclusion**: trichomoniasis in males was considered unimportant, with the belief that it would improve on its own. This study suggests that parasites can be considered as one of the male infertility factors, however, the impact is not fully understood. This investigation found that trichomoniasis can decrease the functional sperm quality.

Keywords: *Trichomonas vaginalis*, fertility and infertility, diagnosis, PCR, men Correspondence Email(s): f.foroughi@umsha.ac.ir Poster

Frequency of *Argas* infestation in dovecotes of Bam city

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**Background**: The pigeon tick, *Argas reflexus*, is a significant ectoparasite of pigeons. These ticks can migrate into human dwellings when they lose their natural pigeon hosts. Their bites can cause severe health issues. Repeated *Argas* bites can lead to allergic reactions, which in extreme cases, may result in life-threatening symptoms.

**Methods:** This study conducted from October to December 2023, relied on interviews with pigeon breeders and pigeon fanciers in Bam city, Kerman province. The interview process commenced by identifying the pigeon breeders and pigeon fanciers, followed by online interviews. They were queried about *Argas* species infestation in their dovecotes. Images and basic information were provided for those who did not know the *Argas* ticks so they could answer the questions properly.

**Results**: A total of sixty-four pigeon breeders and pigeon fanciers participated, twenty-five respondents did not provide answer to questions, and twenty-nine dovecotes were free from Argas. The results revealed that ten dovecotes were infected with *Argas* spp.

**Conclusion**: The management of dovecotes plays a crucial role in preventing *Argas* species infection. Interestingly, while some pigeon fanciers have never encountered this parasite, others consider it common, especially during warm seasons. The blood-sucking ability of this parasite underscores its medical significance.

Keywords: Pigeon, Argas reflexus, infestation, public

Frequency of ectoparasites of domestic pigeons (*Columba livia domestica*) in Mashhad city, Iran

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**Background**: Ectoparasites of pigeons, which cause different diseases in their hosts, are of medical and veterinary importance. These parasites adversely affect growth, productivity and welfare of pigeons. Some of them migrate to human buildings and attack humans. This study aimed to investigate the frequency of ectoparasites of domestic pigeons in Mashhad.

**Methods:** This study was conducted from January to March 2024 in Mashhad city located in northeastern Iran. Seventy-nine domestic pigeons (*Columba livia domestica*) from various private facilities in different areas of the city were randomly selected and examined for ectoparasites, visually. Retrieved parasites were collected and preserved in 70% ethanol before being cleared and mounted on microscopic slides.

**Results**: From 79 pigeons examined, six (7.59%) were found to be infested. The most frequent ectoparasites was *Columbicola columbae* that observed on 2.37% of examined birds followed by *Pseudolynchia canariensis* with abundance of 1.58%.

**Conclusion**: The results of this study showed that examined pigeons were infested with lice and blood-feeding hippoboscid flies. Therefore, husbandry practices should be improved and control measures need to be taken seriously.

**Keywords**: Pigeon, parasite, Mashhad, lice, *Pseudolynchia* 

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Poster

Frequency of sarcocystosis and cysticercosis in slaughtered livestock in abattoirs of Golestan province from 2011 to 2021

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**Background**: Sarcocystosis and cysticercosis are important zoonosis parasitic disease with a high economic and public health impacts. The aim of this study was to determine the frequency of sarcocystosis and cysticercosis in the livestock slaughtered in the abattoirs of Golestan province during 2011- 2021.

**Methods:** This was a retrospective descriptive cross-sectional study. The studied population included all the animals slaughtered in the abattoirs of Golestan province between April 2011 and March 2021. The data on the infectin of livestock with *Sarcocystis* and *Cysticercus* parasites was obtained from the General Veterinary Department of Golestan province. To compare the parasitic infections among the study groups, independent t-tests and analysis of variance were used, considering the normality of the data distribution. All statistical tests were performed using SPSS software version 25, with a significance level of P0.05.

**Results**: During the 11-year period, 2,383,820 livestock, including 154,540 cows, 1,921,595 sheep, and 307,685 goats, were slaughtered in the abattoirs of Golestan province. In total, 528 animals (0.022%) had *Sarcocystis*, 2665 animals (0.111%) had hepatic cysticercosis, and 2074 animals (0.087%) had pulmonary cysticercosis. *Sarcocystis* prevalence was higher in sheep than other animals (P-value 0.001). No significant difference was observed in terms of the frequency of *Sarcocystis* across different years. Comparing the frequency of cysticercosis in liver and lung, no significant difference was observed No significant association was found among cattle, sheep and goats, according to the organs infected with cysticercosis

**Conclusion**: Based on the results and the level of infection in the slaughtered livestock, hygiene-related measures are recommended to reduce infection in livestock, as well as to raise public awareness about the importance of fully cooking meat and meat products to prevent human infection.

Keywords: sarcocystosis, cysticercosis, abattoir, Golestan, Iran

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Frequency of success, treatment failure, and clinical resistance in patients with cutaneous leishmaniasis referred to Leishmaniasis Reference Laboratory of Dezful University of Medical Sciences during 2021-2022

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**Background**: Leishmaniosis, due to the complexity of disease transmission dynamics and significant climatic changes, ranks among the most important health challenges in Iran today. Dezful County is also one of the hotspots for this disease in Iran. Patient monitoring during treatment plays a crucial role in the success of treatment.

**Methods:** A demographic and epidemiological questionnaire was administered to each patient, gathering information on gender, age, place of residence, nationality, disease onset time, accompanying symptoms, medical history, and previous infections. Additionally, data on standard anti-leishmanial treatment regimen, lesion diameter, location, number, shape, and duration, parasitological laboratory results, treatment regimen, dosage, duration, and treatment outcome were collected and recorded for each patient in a checklist format.

**Results**: The results of this study indicated that out of 296 cases of the disease, 105 cases were female and 191 cases were male. The average age of disease occurrence in both sexes was  $26.55 \pm 18.72$ ,  $27.16 \pm 17.77$  in men and  $25.45 \pm 20.39$  in women. In terms of permanent residence of patients, 141 patients (47.5%) lived in rural areas and (52.5%) 156 lived in urban areas. Regarding treatment response, two cases of relapse and three cases of treatment failure were reported, while 291 cases showed improvement with the standard national cutaneous leishmaniasis treatment.

**Conclusion**: This study demonstrated instances of treatment failure and disease relapse during the treatment process. Therefore, meticulous patient monitoring during treatment and evaluating treatment response at regular intervals can alter the disease outcome. The results of this research underscore the necessity for further studies, such as investigating cases resistant to treatment,

**Keywords**: Cutaneous leishmaniasis, Dezful, recurrence, treatment

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Poster

Frequency study of the soil contamination to *Toxoplasma gondii* oocysts by concentration and molecular method in Shiraz, southern Iran

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**Background**: Soil contamination with *Toxoplasma* oocysts, is one of the most important risk factors of human infection, especially in children due to their behavior, the purpose of this study is to determine the frequency of soil contamination with *Toxoplasma gondii* oocysts by concentration and molecular methods in Shiraz county.

**Methods:** samples were obtained from 11 distrinc of Shiraz and Sadra and Golestan towns once in each season. After sample collection, the contamination of soil with *T. gondii* oocysts were investigated by sodium nitrate flotation method and microscopic examination. Also, the prevalence of contamination was done with the Nested-PCR method.

**Results**: In this study, 312 soil samples were collected from the parks of 13 geographical location in Shiraz city and Sadra and Golestan towns during 2019. After flotation with sodium nitrate and microscopic examination, 15 samples (4.8%) were positive. The results of Nested-PCR showed that in addition to 15 samples containing *T. gondii* oocysts that were diagnosed positive by microscopic method, 6 other samples also contained parasite DNA (6.73%). The prevalence of infection was significantly higher in spring season than other seasons (P = 0.01).

**Conclusion**: In this study, the relationship between *Toxoplasma* and soil as an important epidemiological source and one of the important risk factors of toxoplasmosis was investigated and new information was presented about the parasite situation in soil and different areas of Shiraz county.

**Keywords**: Toxoplasma, soil, Shiraz, flotation, Nested-PCR

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GAPDH analysis: a new method in detection of Leishmania major and Crithidia

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**Background**: *Leishmania* and *Crithidia* are members of Trypanosomatidae family that cause diseases in vertebrates and insects, respectively. Recently, studies reported *Crithidia* from the clinical samples of cutaneous leishmaniasis. Therefore, introducing a sensitive and specific method for differentiation of these two genuses is critical.

**Methods:** *L. major* and *Crithidia* isolates were used for setting up the method. The sequences of GAPDH from *L. major* and *Crithidia* were obtained from GenBank analyzed using multiple alignments. RNA extraction and cDNA synthesis was done by the related kits based on the instructions' Company. The specific primer pair was designed. The conventional PCR was performed and the amplicons were assessed using gel agarose electrophoresis. SYBR Green Real Time PCR was done and melting curves were analyzed for detecting Tm.

**Results**: The results of conventional PCR showed the primers were friendly with the fragment of 200 bp in length. Tm from GAPDH of *L. major* and *Crithidia* were 81.25 and 83.34 °C, respectively.

**Conclusion**: Based on knowledge, it is the first report for introducing a useful method for differentiation of *Crithidia* and *L. major* 

**Keywords**: *Leishmania*, *Crithidia*, glyceraldehyde-3-phosphate

**Correspondence Email(s):** eslami\_g2000@yahoo.com Poster

Gastrointestinal nematodes in sheep and goats of Darab

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**Background**: Gastrointestinal parasites in sheep reduce economic production and transmit contamination to humans. This study investigated the prevalence of gastrointestinal nematodes in sheep and goats in Darab using the flotation method. The descriptive cross-sectional study took during early autumn and the beginning of the rainy season in 1402.

**Methods:** Samples were collected from 55 sheep and 55 goats from 10 herds, with 11 samples taken randomly from each herd. Then the samples were transferred to the laboratory and the helminth parasites were isolated using the fecal flotation method, and identify helminth egg parasites (Soulsby, 1982), finally, the obtained data were statistically analyzed.

**Results**: The study revealed a 38.2% prevalence of parasitic infection in the sheep population, with *Marshallagia* being the most common nematode at 48.2%, followed by *Nematodirus* at 22.7%, *Stratagia* at 15.5%, and *Trichostrongylus* and *Haemonchus* at 8.2% each.

**Conclusion**: Age significantly influenced the prevalence of gastrointestinal nematodes, with older sheep showing higher infection rates than younger ones. Livestock type and gender did not affect the prevalence of these parasites. The study underscores the importance of implementing control, mitigating economic losses, and preventing the transmission of infections to humans.

Keywords: Gastrointestinal parasites, Marshallagia, Nematodirus, Darab

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Alborz University of Medical, Sciences, Karaj, Iran May 21 - 23, 2024

Poster

Genetic and phylogenetic evaluations of Schistosoma *turkestanicum* isolated from goats raised in western Iran

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Background: Schistosomiasis, as one of the common zoonotic parasitic infections, is the cause of serious problems in the animal husbandry industry, which can impose heavy economic losses. However, there is limited information regarding the types of Schistosoma (= Orientobilharzia) turkestanicum genotypes in Iran.

Methods: For this purpose, after extracting DNA from several male parasitic worms, the mitochondrial Cox1 gene was proliferated using a polymerase chain reaction (PCR) assay and sent for sequencing after ethanol alcohol purification. Subsequently, after editing the sequence using the CLC Main Workbench, the neighbor-joining procedure with 1000 bootstraps from MEGA6 software was employed to plot a phylogenetic diagram to assess the evolutionary relationships between the Cox1 gene sequence identified in this study and various biological species of S. turkestanicum collected from NCBI. The CLC Main Workbench was also used to plot the genetic distance matrix (nucleotide differences and similarities).

Results: Based on the phylogenetic analyses, the investigated parasite in this study belonged to the genus Schistosoma.

Conclusion: According to the present findings, the lineage of S. turkestanicum was very close to S. turkestanicum identified in Mazandaran province, as well as the African Schistosoma species found in other regions.

Keywords: Schistosoma, goat, polymerase chain reaction

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Poster

Genetic profile of *Echinococcus granulosus* isolated from the livestock and human in northwest Iran

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Background: Echinococcosis is categorized among the highly important zoonoses, occurring in many regions of Iran, and Iran a country considered as a significant endemic focus of cystic hydatid disease with major economic and public health concern in the country. The current study aimed to identify the genotype and species profile

Methods: A total of 80 Cysts were obtained from farm animals including sheep, cattle, and goats, and the humans. DNA extraction and PCR-sequencing were conducted targeting the mitochondrial CO1 and ND1 regions. Molecular phylogeny and haplotype network analysis was calculated for the isolates.

Results: Out of 79 evaluated samples, 97.5% belonged to G1 genotype and 2.5% to G3 genotype. The G1 genotype was present in all infected participants. Also, the study showed the G1 to be the most common genotype of E. granulosus among both the humans and the animals examined.

**Conclusion**: The study demonstrated the presence of hydatid cysts in human and livestock caused by two highly identical genotypes known as G1 and G3 within E. granulosus sensu stricto. More molecular and haplotype analysis studies are required to identify the contamination origin and the dynamics influencing the transmission process of

Keywords: Echinococcus granulosus, COX1, NAD1, phylogenetic

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Genetic variation in mitochondrial COX1 and Ribosomal ITS2 genes of *Toxocara canis* in stray dogs in Zabol, southeast Iran

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**Background**: Toxocariasis is a zoonotic parasitic disease with worldwide distribution caused by the larval stage of ascaridoid nematodes of dogs (*Toxocara canis*) and cats (*Toxocara cati*). The study was accomplished to determine the sequence variation in ITS2 and COX1genes within isolates of *T. canis*.

**Methods:** Two hundred Stool samples were collected randomly from dogs in public parks and streets from different regions of Zabol. Thirty samples containing eggs were isolated from the feces using formalin ether 10% and centrifugal flotation. Genomic DNA was extracted, and COX1 and ITS2 were amplified by PCR-RFLP and sequenced. Sequence data were aligned using the BioEdit software and BLAST program and compared with published sequences in GenBank. The phylogenetic relationship between isolates of T. canis from Zabol city with other regions based on sequences obtained from COX1 and ITS2 genes and using MEGA7.0 software was investigated.

**Results**: For all samples, amplicons of about 388 and 422 base pairs were produced by PCR for ITS2 and COX1, respectively. Drawing the phylogenic tree of ITS2 and COX1 sequences of isolates of *T. canis* showed that the identified genotypes are not only different from each other but also from other parts of the world.

**Conclusion**: Our result showed that genetic variation among isolates of *T. canis* from Zabol is very low. For a deeper understanding of genetic diversity among populations of *Toxocara*, it is recommended to analyze more isolates from various geographical areas and variable genetic markers.

Keywords: Genetic variation, ribosomal DNA, Toxocara

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Poster

Genotyping of *Blastocystis* sp. in children using PCRsequencing method in southeast Iran

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**Background**: *Blastocystis* sp is a zoonotic anaerobic parasite in the large intestine of humans and many vertebrates. It is mostly found in people who are in contact with animals. Current study aimed at identifying the prevalence of *Blastocystis* sp. and its common genotypes in children with clinical symptoms of diarrhea

**Methods:** A cross-sectional descriptive study was conducted on 294 children under ten years of age with gastrointestinal symptoms, especially diarrhea. After sample collection, stool samples were subjected to direct stool testing for initial diagnosis. After microscopic diagnosis, DNA extraction and PCR test with small subunit ribosomal RNA (SSU rRNA) gene target were performed, and PCR products were purified and sequenced. Nucleotide sequences were revised using Chromas biotechnology software version 2.4 and CLC genomic work bench software 11. Nucleotide sequences were aligned using BLAST database and compared with reference genotypes of *Blastocystis* sp in gene bank. Genotyping of sequences

**Results**: Out of 294 examined cases, 5 children (1.7%) were positive by direct microscopic and PCR tests, where a 500 (479) bp fragment in the SSU-rRNA target was detected. Genetic analysis identified 4 subtypes, including subtypes 1, 2, 3, and 5.

**Conclusion**: Considering the presence of subtypes 3 and 5 in the study and the evidence of their zoonosis, examining the parasite dynamics and epidemiological principles can be effective in the control strategy.

Keywords: *Blastocystis* sp, children, PCR-sequencing, southeast

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Genotyping of *Trichomonas vaginalis* isolated from pregnant women in Mazandaran province using Nested PCR

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**Background**: Trichomoniasis is one of the most important parasitic infections of the lower genitourinary tract in men and women. Therefore, the purpose of this research is to investigate the prevalence of trichomoniasis in women referring to health clinics in Mazandaran province and to investigate the genotyping of *Trichomonas vaginalis* parasite.

**Methods:** In this cross-sectional descriptive study, 612 vaginal swab samples were collected between January 2021 and March 2022. The samples were first examined using the direct method, and then they were cultured in TYM culture medium, and the positive samples were kept at -20 temperature until molecular tests were performed. All information was compared using Chi-2 test with a confidence level of 95% and P-value less than or equal to 0.05 was considered statistically significant.

**Results**: The results of this study showed that using the direct method, all the samples were reported negative, and using the culture method in TYM medium, 6 samples were positive. Positive samples were evaluated using the Nested PCR molecular test, and 4 samples were positive. Out of the 4 examined samples, 3 samples have been successfully sequenced and all isolates were related to *T. vaginalis* genotype G. It has also been determined that there was no significant relationship between the incidence of trichomoniasis and the age and place of residence of the patients.

**Conclusion**: The results of this research have shown that 98% of the referring pregnant women were positive for trichomoniasis and the sensitivity of the cultivation method is much higher than the direct method.

Keywords: Actin, genotype G, Nested PCR

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Poster

Giardia cyst in the water of Kan River in Tehran

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**Background**: *Giardia* is the most important causal agent of non-bloody diarrhea in many countries including Iran. Many rivers are contaminated with *Giardia* cysts because of domestic wastewater or farms wastewater and the living of rodents on their margins. The present study aims to evaluate Kan River contamination with *Giardia* cyst.

**Methods:** Sampling was conducted from different parts of Kan River in different seasons in 2019. First, the smear has been prepared from sediments after filtering the water and collecting the sediment, and stained with trichrome and finally were examined microscopically. Then, they were amplified with Nested-PCR method by using the specific primers, the giardian gene from Giardia. Positive samples were sequenced and phylogenetic tree was drawn.

**Results**: After the microscopic investigation, 12 suspected samples of *Giardia* cyst were detected, but only 4 samples infected with Giardia were molecularly found. In terms of genotype, the identified *Giardia* was 100% consistent with human isolates of assemblage B of *G. duodenalis*.

**Conclusion**: The presence of *Giardia* cyst in the water of Kan River indicates the contamination of this river to a pathogenic human parasite. Assemblage B as the dominant *G. duodenalis* genotype, is commonly reported in human and only a small number of animal species.

Keywords: Giardia, Kan River, Tehran

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#### Poster

Granzyme A expression level in NK cells exposed to *Toxoplasma* gondii

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**Background**: Natural killer (NK) cells use the granzyme pathway as a major mechanism to kill pathogen-containing cells; granzyme A (GZMA) expressions as markers of NK cell-stimulating against *Toxoplasma gondii* (*T. gondii*) infection. We aimed to assessed the expression level of GZMA in NK cells exposed to *T. gondii* tachyzoites in laboratory.

**Methods:** In the current in vitro designed study, we conducted an in vitro experiment to evaluate NK cell differentiation and activation from human umbilical cord blood mononuclear cells (UCB-MNCs) after infection with *T. gondii* tachyzoites. UCB-MNCs were infected by fresh tachyzoites of type I (RH) or type II (PTG) strains of *T. gondii* pre-expanded in mesenchymal stem cells for two weeks in a medium enriched with stem cell factor (SCF), Flt3, IL-2, and IL-15. Flow cytometry analysis were performed, as well, we measured protein levels of granzyme A expressions by western blotting.

**Results**: We found that exposure of UCB-MNCs with tachyzoites lead to a decrease of GZMA compared to the MNC non-treated control group. Data revealed that incubation of UCB-MNCs with NK cell differentiation medium increased the GZMA. Noteworthy, two-week culture of UCB-MNCs with type I (RH) tachyzoites significantly suppressed GZMA, showing reduction of NK cell differentiation from cord blood cells.

**Conclusion**: Our findings suggest that virulent *T. gondii* tachyzoites diminished granzyme A synthesis in UCB-MNCs and with cytopathic effects inhibit NK cell activation and eliminates innate immune responses during infection; and consequently, enable the parasite to continue its survival in the host body.

Keywords: Toxoplasma gondii, granzyme A, umbilical

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Poster

Green synthesized zinc nanoparticles as a potential control agent of *Leishmania major* infection

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**Background**: We decided to investigate the anti*Leishmania*l, cellular mechanisms, and cytotoxic effects of green synthesized zinc nanoparticles (ZnNPs) alone and combined with glucantime against *Leishmania major* infection.

**Methods:** The effect of green synthesized ZnNP on *L. major* amastigote was studied through macrophage cells. The mRNA expression level of iNOS and IFN- $\gamma$  followed by the exposure of J774-A1 macrophage cells to ZnNPs was assessed by Real-time PCR. The Caspase-3-like activity of promastigotes exposed to ZnNPs was studied. Effects of ZnNPs alone and combined with glucantime (MA) were studied on cutaneous leishmaniasis in BALB/c mice.

**Results**: ZnNPs displayed the spherical shape with sizes ranging from 30 to 80 nm. The obtained IC50 values for ZnNPs, MA, and ZnNPs + MA were 43.2, 26.3, and 12.6  $\mu$ g/mL, respectively; indicating the synergistic effects of ZnNPs in combination with MA. CL lesions had completely improved in the mice received with ZnNPs in combination with MA. The mRNA expression level of iNOS, TNF- $\alpha$ , and IFN- $\gamma$  was dose-dependently (p 0.01) upregulated; whereas it was downregulated in IL-10. ZnNPs markedly stimulated the caspase-3 activation with no significant toxicity on normal cells.

**Conclusion**: Based on these in vitro and in vivo results, green synthesized ZnNPs, mainly along with MA, showed that has the potential to be introduced as a new drug for CL therapy. Triggering of NO production, and inhibition of infectivity rate are revealed as mechanisms of action ZnNPs on *L. major*.

Keywords: Leishmaniasis, nanomedicine, in vitro

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High potency of *Capparis spinosa* extract to inactivate protoscoleces during hydatid cyst surgery

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**Background**: Hydatidosis is one of the most dangerous zoonosis diseases in the world caused by the larval stage of the broad-worm or *Echinococcus granulosus* parasite. Today, cysts' rupture or content leakage during surgery and involvement of organs adjacent to the organ involved, and consequently secondary cysts.

**Methods:** Collected protoscoleces from liver fertile hydatid cysts of infected sheep were exposed to the different concentrations of the essential oil (150, 300, 600 mg/mL) for 5-60 min in vitro and ex vivo. Then by using the eosin exclusion assay, the viability of protoscoleces was studied. The primary phytochemical analysis of the *C. spinosa* extract was done to assess the presence of tannins, alkaloids, saponins, flavonoids, terpenoids and glycosides.

**Results**: C. spinosa extract exhibited a powerful protoscolicidal activity in vitro so at the dose of 300 and 600 mg/ml, it entirely eliminated the parasite after 10 and 5 minutes; whereas at lower doses, it demonstrated weak protoscolicidal activity. In ex vivo assay, no similar effect to in vitro assay was observed, so more time was required to show a potent protoscolicidal activity. *C. spinosa* extract, at the concentrations of 300 and 600 mg/mL after an exposure time of 20 and 12 min, killed 100% of protoscoleces within the hydatid cyst, respectively. The findings of primary phytochemical screening of the *spinosa* extract.

**Conclusion**: The obtained results in vitro and ex vivo exhibited potent protoscolicidal effects of C. spinosa extract particularly at the concentrations of 600 and 300 mg/mL, which entirely eliminated the parasite after 5-20 min exposure. However, more supplementary works are required to verify these findings through assessing in animal models.

 Keywords: hydatidosis, herbal medicines, scolicidal, extract
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Poster

High-resolution melting analysis in comparison with microscopic method: an experimental study to diagnosis of *Plasmodium* species infections in human

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**Background**: Among the human parasitic diseases, malaria is the main cause of morbidity and mortality. To prevent the high mortality and tracking malaria elimination efforts, a prompt and sensitive diagnosis is essential. This study aimed to compare High-Resolution Melting (HRM) and microscopic methods to diagnose *Plasmodium falciparum* and *P. vivax*.

**Methods:** Eighty-one blood samples were collected from patients with clinical symptoms who were suspected of malaria in Chabahar district, southeastern Iran and also from those who were referred to Malaria National Laboratory in the Tehran University of Medical Sciences, Tehran, Iran. Microscopic examination and HRM method were used to diagnose *Plasmodium* parasites simultaneously.

**Results**: Microscopic results revealed 45 positive cases (12 *P. falciparum* and 33 P. vivax) out of 81 collected samples. According to the results of the HRM analysis, 11 and 33 samples were identified as *P. falciparum* and *P. vivax*, respectively. HRM analysis also revealed 1 mixed infection of *P. falciparum* and *P. malariae*.

**Conclusion**: HRM analysis provides a promising mean for simultaneous detection and discrimination of the *Plasmodium* spp. especially in mixed infection cases.

**Keywords**: Malaria, diagnosis, microscopy, *Plasmodium* 

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Histological study of the interaction effect of New Zealand rabbit earlobe blastema tissue and 3D decellularized scaffold of earthworm middle part in vitro

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**Background**: The extracellular matrix contains a complex set of structural and functional proteins that play an important role in tissue morphogenesis, migration, proliferation and wound healing. The aim of this research was the mutual effect of blastma tissue obtained from rabbit earlobe punch wounds and decellularized scaffold of earthworm forming tissues.

**Methods:** First, the earthworms were divided into smaller pieces. Then, using physical methods (repeated freezing and thawing) and chemical methods (use of ionic detergent sodium dosyl sulfate (SDS)) in different concentrations and times, the resulting tissues were decellularized. Also, two days after the ear punching of New Zealand male rabbits, their blastema tissue was separated in the form of a ring. The blastema ring was transferred to the decellularized earthworm scaffold and kept in the culture medium for 40 days then, the relationship between the blastema tissue and the elastic scaffold was examined every 10 days.

**Results**: The results showed the penetration of blastema tissue cells and the destruction of a part of the earthworm's threedimensional scaffold during the penetration, proliferation and possible secretion of cells. Also, the best time for the penetration of cells in the scaffold was on days 4 and 5 after cultivation.

**Conclusion**: This study showed that the decellularized earthworm matrix probably has inducing effects on the migration of blastema tissue cells and can be suggested as a natural scaffold for possible applications in future research projects.

Keywords: Bio scaffold, earthworm, blastema tissue

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Poster

Identification and characterization of free-living amoeba in contact lens washing solutions and hospital environments in the ophthalmology wards and operating rooms in southeastern Iran

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**Background**: Among the free-living amoeba, different species of *Acanthamoeba* are the most ubiquitous organisms causing serious diseases in humans including CNS, cutaneous and eye infections. Very few studies have been conducted in the hospital environments particularly the environments of ophthalmology wards where people are present with different types.

**Methods:** Samples were collected from the used contact lenses and their washing solutions, and from indoor and outdoor environmental samples from the ophthalmology ward and operating room of Shafa Medical Center, a major referral hospital in Kerman, Southeastern Iran. The samples were cultured on non-nutrient agar (NNA) in 28-30oC and microscopically studied. Molecular study including PCR-sequencing and phylogenetic analysis on partial 18S rDNA were performed on positive culture samples.

**Results**: In total 70 samples were collected from the patients and hospital environment of which 11.4% (8 out of 70) were found positive using NNA culture. Acanthamoeba cysts were identified in two out of 22 dust samples (9.1%) collected from the ophthalmology ward and operating room. No samples from the washing solutions were found contaminated, however two samples out of the four used contact lenses were positive for the presence of *Acanthamoeba* T4 genotype. *Protacanthamoeba bohemica* were found in the soil samples from outdoor environment of the ophthalmology ward in the hospital.

**Conclusion**: This study identified *Acanthamoeba* species in contact lenses, their storage solutions and the environmental samples from the ophthalmology ward. T4 genotype, which is the most abundant etiology of *Acanthamoeba keratitis*, was found in this study.

Keywords: Dust, contact lens; *Acanthamoeba*, *Protacanthamoeba* Correspondence Email(s): majid.fasihi@gmail.com 5th International & 12th National Congress of Parasitology and Parasitic Diseases of Iran Alborz University of Medical, Sciences, Karaj, Iran

#### Poster

Identification of medical-important viral and fungal endosymbionts in free-living amoebae isolated from hospital wastewater facilities; a critical public health alarm

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**Background**: Free-living amoebae (FLA) are amphizoic microorganisms, isolated from environmental sources. In this study, we investigated fungal and viral endosymbionts in FLA isolated from hospital wastewater treatment systems.

**Methods:** 104 samples were collected from the wastewater facilities of two, one, and one general, children, and women hospitals in Tehran for 7 months. Samples were filtered, cultivated, and microscopically monitored. After purification of FLA, DNA and RNA extraction was carried out. Identification of FLA was performed using amplification and sequencing of target genes. The presence of viral (Norovirus, Adenovirus, Rotavirus, and Astrovirus) and fungal (*Mucor* spp., *C. albicans, A. flavus*, and *Fusarium* spp.) was evaluated using real-time PCR. For RNA viruses, cDNA synthesis was performed before PCR amplification.

**Results**: Out of 104 wastewater samples 94 (97.7%) were microscopically positive for FLA. From 94 FLA positive samples, 59 (63.76%) plates were purified and included in molecular assays. The presence of at least one of FLA was confirmed in 59 samples. Accordingly, 52, 5, and 10 samples were positive for Acanthamoeba sp., Vermamoeba spp., and Vahlkampfiidae, respectively. Viral endosymbionts were detected from seven wastewater samples (four inlets and three outlets) including seven, three, and one of Norovirus, Astrovirus, and Adenovirus, respectively. *Fusarium* spp., were detected in eight samples (four for each inlet and outlet).

**Conclusion**: Regarding the capability of FLA to carry potentially pathogenic endosymbionts and their ability to survive harsh condition such as normal wastewater treatment, the presence of FLA in hospital wastewater may increase the risk of transmission of potentially pathogenic microorganisms to downstream farmlands that use treated wastewater for irrigation.

**Keywords**: wastewater; FLA; Viral-endosymbionts; Fungalendosymbionts. **Correspondence Email(s):** 

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Poster

May 21 - 23, 2024

Identification of potential inhibitors for Nmyristoyltransferase (NMT) protein of *Leishmania* spp. through in silico approaches

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**Background**: Leishmaniasis is a parasitic disease found in subtropical, tropical, and Southern Europe. Leishmaniasis treatment is a complicated topic. Despite being limited, the current treatments are toxic and have side effects. Also, most of the time, they cannot treat the resistant form of *Leishmania* parasites.

**Methods:** From the PubChem database, the 3D structures of the N-myristoyltransferase (NMT) protein from *Leishmania major*, as well as blockers and 2000 herbal compounds from 150 herbs, were retrieved. utilizing PyRx software and AutoDock vina, a molecular docking analysis was conducted against *Leishmania* protein individually utilizing herbal drugs and proteins blockers. The activity, daily carcinogenicity, and ADMET characteristics derived from Swiss ADME, Lazar, and way 2 drug. Molecules with the greatest docking scores for protein were chosen for molecular dynamic simulation using the GROMACS program version 5.

**Results**: According to the findings of molecular docking experiments, hypericin have a strong affinity for the NMT protein.

**Conclusion**: According to information gathered from pharmaceutical databases, the mentioned substance may have antiinflammatory and wound-healing properties in addition to blocking proteins. Therefore, experimentally examining these compound could be a valuable clue to the control and treatment of leishmaniasis.

Keywords: Leishmaniasis, molecular dynamics, herbal compounds Correspondence Email(s): hejazi@med.mui.ac.ir



Identification of species of Lishmania Isolates from patients with suspected Cutaneous *Leishmanias*is in Shushtar City, Southwestern Iran

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**Background**: Cutaneous leishmaniasis (CL) is a health problem of many countries. There is in the most cities of Iran. This study aimed to investigate on species of *Lishmania* isolates from patients with suspected cutaneous leishmaniasis referring to Health Center of Shushtar, Khuzestan province, southwest of Iran in during 2021 to 2022.

**Methods:** In this study was carried out by preparing slides from skin lesions of 78 patients suspected to leishmaniasis. Direct microscopy and polymerase chain reaction (PCR) performed using specific kinetoplast DNA primers. Data were analyzed with SPSS.

**Results**: Among 78 persons with skin ulcers suspected to CL, the results of direct smear of 26 (33.33%) of examined samples were positive. PCR band were observed in 36 (46.1%) of examined samples in which all banding patterns were diagnosed as *L. major* in compare with standard models with amplified fragments 560 bp in length from bands. The patients were 61.53% male and 38.46% female. The most frequently-infected age group was the 20-30 years. The significant correlation was observed between gender and the number of lesion (P < 0.05).

**Conclusion**: Molecular analysis revealed that dominant species isolated from Shushtar were identified as *L. major*. With considering the relatively high statistics of contamination, reservoir of rodents and sand flies, can be significant in the occurrence of leishmaniasis so further study is necessary to discover that *L. major* is the etiologic agent.

Keywords: Cutaneous leishmaniasis, Iran, Leishmania parasites

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Poster

Identification tick vector of *Hepatozoon* canis in dogs in Khorasan Razavi province, Iran

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**Background**: *Hepatozoon canis* is a haemoprotozoan in domestic and wild dogs that is transmitted by *Rhipicephalus* spp. A survey was carried out to identify the tick vector of *H. canis* in dogs in the Mashhad area, Khorasan Razavi province from 2020 to 2021.

**Methods:** A total of 976 ixodid ticks were collected from 39 sheepdogs and 76 shelterdogs during activating seasons of ticks. Adult ticks were identified according to general identification keys. The collected female ticks were separated into 81 tick pools according to their species. The hemolymph smear was prepared from engorged and semi-engorged female ticks and stained with the Giemsa method, Then, the DNA of each tick pool was extracted using a commercial kit and analyzed by PCR. Two ixodid species, *Rhipicephalus sanguineus* and *Rhipicephalus turanicus* were identified in infested dogs.

**Results**: The frequency of R. sanguineus and *R. turanicus* infestation in sheepdogs was 80.25% and 19.75% and in shelterdogs 76.5% and 23.5%, respectively. *H. canis* DNA has only been detected in 15 (18.5%) tick pool samples of *R. sanguineus*. The *Hepatozoon* oocysts were observed in two hemolymph smears of engorged female *R. sanguineus* that were collected from shelterdogs.

**Conclusion**: It is concluded that *R. sanguineous* was the dominant tick in sheepdogs and shelter dogs in the Mashhad area. Moreover, the results of the molecular and parasitological examination indicated that *R. sanguineus* ticks could be a vector of *H. canis* in dogs in Iran for the first time.

Keywords: Ticks, vector, Hepatozoon, dogs, Iran

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Immunogenicity investigation of microwave treatment on Toxoplasma gondii tachyzoites of RH strain by in vitro and in vivo methods

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**Background**: *Toxoplasma gondii* is a ubiquitous protozoan parasite. It poses severe consequences for vulnerable individuals. It has no definitive treatment. Vaccination is the best preventive measure. Yet an effective human vaccine is lacking. Therefore, evaluating the immunogenicity of microwave-treated tachyzoites and suggesting a potential strategy for vaccine development are crucial.

**Methods:** Tachyzoites of RH strain of *Toxoplasma* were treated with high-power microwave oven for 5, 10, 15, and 20 seconds. Subsequently, for viability assessment, the treated tachyzoites were examined using flow cytometry. The in vitro infectivity power of tachyzoites was investigated in HeLa cell culture. For in vivo study, the suspension of treated tachyzoites was inoculated twice into BALB/c mice with one-month interval. Then, the immunity conferred to surviving mice was assessed by inoculating untreated tachyzoites. Additionally, blood sampling was conducted at various stages, and antibody titers produced in surviving mice were measured using the MAT serology method.

**Results**: In the flow cytometry method, the percentage of death related to mentioned times was 46.86%, 74.11%, 88.38% and 99.34%, respectively. In in vitro examination, no parasite replication was observed at time doses of 10 to 20 seconds. In vivo test results showed that all mice died due to inoculation with tachyzoites treated for 5 seconds, but in groups treated for 10 to 20 seconds, all mice survived. As a result of exposure of surviving mice to untreated tachyzoites, a significant number remained immune (n = 12). The MAT test results showed a titer of 1/16 for the surviving mice.

**Conclusion**: Based on the results obtained from this study, it appears that treating *T. gondii* RH strain tachyzoites with microwave may induce immunity against this parasite in mice. Therefore, this method could be considered as an effective, convenient, and accessible approach for developing a vaccine against *T. gondii* infection.

Keywords: *Toxoplasma gondii*, tachyzoite, immunogenicity, microwave Correspondence Email(s): asgarig@sums.ac.ir qasemasgari@yahoo.com Poster

Immunoinformatics studies and design of a novel multi-epitope peptide vaccine against *Toxoplasma gondii* based on calcium-dependent protein kinases antigens through an in silico analysis

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**Background**: Infection by the intracellular apicomplexan parasite *Toxoplasma gondii* has serious clinical consequences in humans and veterinarians around the world. Although about a third of the world's population is infected with *T. gondii*, there is still no effective vaccine against this disease.

**Methods:** Top-ranked MHC-I and MHC-II binding as well as shared, immunodominant linear B-cell epitopes were predicted and linked using appropriate linkers. Moreover, the 50S ribosomal protein L7/L12 (adjuvant) was mixed with the construct's N-terminal to increase the immunogenicity. Then, the vaccine's physicochemical characteristics, antigenicity, allergenicity, secondary and tertiary structure were predicted.

**Results**: The finally-engineered chimeric vaccine had a length of 680 amino acids with a molecular weight of 74.66 kDa. Analyses of immunogenicity, allergenicity, and multiple physiochemical parameters indicated that the constructed vaccine candidate was soluble, non-allergenic, and immunogenic, making it compatible with humans and hence, a potentially viable and safe vaccine candidate against *T. gondii* parasite.

**Conclusion**: In silico, the vaccine construct was able to trigger primary immune responses. However, further laboratory studies are needed to confirm its effectiveness and safety.

**Keywords**: Immunoinformatics, *T. gondii*, CDPK1, CDPK2,

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In silico and in vitro potentials of crocin and amphotericin B on *Leishmania major*: Multiple synergistic mechanisms of actions

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**Background**: A significant barrier to optimal antileishmanial treatment is low efficacy and the emergence of drug resistance. Multiple approaches were used to monitor and assess crocin (a central component of saffron) mixed with amphotericin B (AmpB) potential in silico and in vitro consequences.

**Methods:** The binding behavior of crocin and iNOS was the purpose of molecular docking. Anti amastigote and Anti promastigotes activity of crocin evaluated in *Leishmania major*.

**Results**: The results showed that crocin coupled with AmpB demonstrated a safe combination; extremely antileishmanial, suppressed *Leishmania* arginase absorption, and increased parasite death. This natural flower component is a robust antioxidant, significantly promoting the expression of the Th1-connected cytokines (IL12p40, IFN- $\gamma$ , and TNF- $\alpha$ ), iNOS, and transcription factors (Elk-1, c-Fos, and STAT-1). In comparison, the expression of the Th2-associated phenotypes (IL-10, IL-4, and TGF- $\beta$ ) was significantly reduced. The leishmanicidal effect of this combination was also mediated through programmed cell death (PCD), as confirmed by the manifestation of phosphatidylserine and cell cycle detention at the sub-GO/G1 phase. In conclusion, crocin with AmpB.

**Conclusion**: The main action of crocin and AmpB involved wide-ranging mechanistic insights for conducting other clinical settings as promising drug candidates for cutaneous leishmaniasis. Therefore, this combination could be esteemed as a basis for a potential bioactive component and a logical source for leishmanicidal drug development against CL in future advanced

**Keywords**: *Leishmania*, crocin, apoptosis, cytotoxicity, immunology

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Poster

In vitro and ex vivo evaluation of the anti-leishmanial activity of emodin herbal extract

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**Background**: Leishmaniasis, caused by the protozoan parasite *Leishmania*, is a global health issue. The search for antileishmanial agents is ongoing, with natural products such as herbal extracts presenting potential therapeutic options. This study aimed to investigate the effect of emodin, a natural herbal extract, on promastigotes and amastigotes of *Leishmania major*.

**Methods:** Different concentrations of emodin (0, 50, 100, 200, and 400  $\mu$ g/mL) were tested, with glucantime used as a positive control at concentrations of 50 and 100  $\mu$ g/mL. The mortality rate of promastigotes was assessed at 24-, 48-, and 72-hours following exposure to emodin and glucantime. Additionally, the MTT assay was performed to calculate the half-maximal inhibitory concentration (IC50) of emodin at each time point. The J774 cell line was utilized to determine the infection rate after 24, 48, and 72 hours of exposure to emodin.

**Results**: The mortality rate of promastigotes at the concentration of 400  $\mu$ g/mL of emodin was higher than that of glucantime at 100  $\mu$ g/mL after 72 hours. Both concentrations of 400  $\mu$ g/mL and 200  $\mu$ g/mL of emodin resulting in 100% mortality rate at this time point. The infection rate after 24, 48, and 72 hours for the concentration of 400  $\mu$ g/mL of emodin did not significantly differ from that of glucantime at 100  $\mu$ g/mL. The calculated IC50 for emodin after 24, 48, and 72 hours was 118.6, 389, and 59.7  $\mu$ g/mL, respectively.

**Conclusion**: The findings demonstrate the potent antileishmanial activity of emodin against *L. major*. Mortality rate and IC50 values indicate the dose-dependent and timedependent efficacy of emodin in inhibiting parasite growth. Further studies is required to evaluate its efficacy invivo, with the ultimate goal of clinical applications for the management of leishmaniasis.

**Keywords**: Emodin, cutaneous leishmaniasis, herbal extract **Correspondence Email(s):** salehisgh@mums.ac.ir



In vitro and in vivo antiparasitic activity of phenolic fraction of *Eryngium caucasicum* extract on RH strain of *Toxoplasma gondii* 

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**Background**: Toxoplasmosis is a common parasitic disease between humans and animals with worldwide prevalence. Eryngium caucasicum is a plant whose anti-parasitic and antimicrobial properties have been proven. This study was conducted with the aim of investigating the anti-toxoplasmic activity of Eryngium caucasicum phenolic fraction on *Toxoplasma gondii* strain RH.

**Methods:** The anti-toxoplasmic activity of different concentrations (1 to 512  $\mu$ g/mL) of *E. caucasicum* phenolic fraction on parasite tachyzoites was investigated in vitro using flow cytometry method. For the in vivo study, mice infected with *Toxoplasma* were divided into 9 groups of 5 each. including negative control, positive control (sulfadiazine 10 mg/kg) and 6 treatment groups with concentrations of 16, 32, 64, 128, 256 and 512 mg/kg. After a few days, the survival time of the mice in the treatment groups was compared with the control groups.

**Results**: The results of flow cytometry showed that the highest percentage of mortality was related to the concentration of 512  $\mu$ g/mL (87.6%). The calculated IC50 was equal to 58.3  $\mu$ g/mL. In the in vivo study, the average survival of mice for the treatment group at the lowest dose (16 mg/kg) was 8.6 days and at the highest dose (512 mg/kg) was 13.6 days.

**Conclusion**: The results of this study showed that *E. caucasicum* phenolic fraction had a high effect on *T. gondii* in in vitro and in vivo environments, which is probably due to the presence of biologically active compounds such as phenol.

**Keywords**: *Toxoplasma gondii*, *Eryngium caucasicum*, phenolic

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Poster

In vitro and in vivo effects of Lufenuron on *Toxoplasma* gondii RH strain.

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**Background**: Lufenuron, a benzoylurea pesticide, inhibits the production of chitin in insects so it used in the veterinary flea control medication program. As *Toxoplasma* tissue cyst wall is composed of chitin fibrils, we decided to investigate the in vitro and in vivo effects of this compound on *T. gondii* RH strain.

**Methods:** After preparation of Lufenuron, 200000 tachyzoites of *T. gondii* strain RH were exposed to the different concentrations (1-512  $\mu$ g/mL) for three hours and then, they were stained by PI and analyzed by FACS. To evaluate the therapeutic quality, 200000 tachyzoites were intradermally inoculated into five mice from each group. The mice were divided into 7 groups including a negative control group, a positive control group and 5 drug groups with doses of 10, 20, 30, 40 and 50 mg/kg. Also an uninfected group only treated by 50 mg/kg. The mice received different concentrations daily by gavage and the longevity were recorded daily.

**Results**: According to the results of flow cytometry, the tachyzoites lethality was 16.5% and 96.8% at 1µg/ml and 512µg/ml concentrations, respectively. IC50 value was calculated 55.82µg/ml. After 14 days of injection, all mice of groups died. The longevity mean of the group treated with 10 mg/kg was equal to 7.8 days whereas this quantity was 11.8 days in the group treated with 50 mg/kg. The longevity mean was 6 days in the negative control while it was more than 20 days in the positive control. Also, this compound had no lethal effect during this test.

**Conclusion**: The results of this study showed the strong lethal effect of Lufenuron on the *Toxoplasma* tachyzoites. In addition, the compound can increase the survival of mice infected with acute toxoplasmosis. It is interesting that dose-response relationships are found in both investigated phases.

Keywords: Toxoplasma gondii, lufneuron, mice

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In vitro and in vivo evaluation of *Frangula alnus* hydroalcoholic extract against *Leishmania major* 

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**Background**: Considering the effectiveness of *Frangula alnus* plant on many microorganisms and the lack of research on the effect of this plant on the *Leishmania*, the present study was conducted in the direction of "the effect of *Frangula alnus* hydroalcoholic extract on the *Leishmania major* in vitro and in vivo.

**Methods:** The chloroform extract of the *Frangula alnus* was prepared, and then, the effect of different concentrations (1-512  $\mu$ g/ml) of the extract on the promastigotes of the *L. major* was investigated using flow cytometry method. Also, after the creation of ulcers in inbred mice, concentrations of 20 and 40 mg/kg of the extract were prescribed by two methods of injection into the wound and local use for 21 days and daily, and the area of the wounds was measured at different times.

**Results**: The results of the direct effect of different concentrations of the extract showed that the concentration of 512 µg/ml had the highest lethality on promastigotes of the *L. major*, which showed a dose-dependent increase. The examination of the wound size in different treatment groups showed that despite the significant increase in the wound area of the mice in the negative control group (P = 0.048), the wound area of the mice in the group receiving the topical extract of this plant with a concentration of 40 mg/kg was Significance has decreased (P = 0.023).

**Conclusion**: Based on the results of this study, the hydroalcoholic extract of *Frangula alnus* has a lethal effect on the promastigotes of *L. major* in a dose-dependent manner, and in mice, when administered topically; it reduces the size of lesions.

Keywords: Leishmania major, Frangula alnus Correspondence Email(s): motazedm@sums.ac.ir Poster

In vitro anthelmintic effect of *Ferula assa-foetida* hydroalcoholic extract against flukes of Fasciola hepatica

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**Background**: With the emergence of drug resistance against the current formulations, there is a need to focus on alternative approaches to control helminth parasites of medical importance. The current study aims to determine the anthelmintic properties of *Ferula assa-foetida* hydroalcoholic extract as an herb in *Fasciola hepatica* treatment, in vitro.

**Methods:** The impacts of various concentrations of *F. assa-foetida* extract at 2000-8000 µg/mL were compared with Triclabendasole (positive control) and RPMI (negative control). Worms were separated exposed to the above extract. Each treatment was repeated thrice at the 5% Co<sub>2</sub> incubator and the number of alive and dead worms was observed at 0, 12, and 24 hours post-treatment. Mortality time was determined by observing the motility of the flukes and examined under the microscope. The anthelmintic efficacy was measured by scanning electron microscopy (SEM) technique. The MTT assay is done to evaluate the cell viability of all cells in culture media.

**Results**: Our experiment by MTT assay on *F. hepatica* showed, thatat concentrations of 8000  $\mu$ g/mL of hydroalcoholic extract of *F.assa-foetida* 3% of the Hella cells were alive, thus the percent of toxicity was 97% in 24 hours. SEM images of treated worms by F.assa-foetida extract (8000  $\mu$ g/mL) showed severe damage, including complete loss of sensory papillae and destruction of prominent network structures and tegument vesicles. The mortality rates and the anthelmintic properties of *F. assa-foetida* highly relied on time and concentration, as far as increasing the time and concentration increasing the mortality rate.

**Conclusion**: It could be concluded that *F. assa-foetida* per formed anthelmintic properties effects. To the best of our knowledge, no previous reports have assessed the effect of *F. assa-foetida* on liver flukes *F. hepatica*. Therefore, the present study provides a basis for future research on the control of these trematodes.

Keywords: Fasciola hepatica, in vitro, Ferul aassa-foetida Correspondence Email(s): arbabi4.mohsen@yahoo.com



In vitro anti-leishmanial effect of local *Scrophularia bavanatia* extract on *Leishmania* (L.) [MHOM/NADIM3] *tropica* promastigotes

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**Background**: Although WHO has recommended glucantime as a cure for CL; yet, there exists some constraints in the use of this remedy such as unexpected complications and high costs. The urgent need for frequent injections, and unsatisfactory efficacy.

**Methods:** *Leishmania* (L.) *tropica* were cultured. Then, the *Scrophularia bavanatia* were provided, sterilized, and added to cultures. However, Then, the proper densities of *S. bavanatia* extract were provided, sterilized, and added to cultures containing the parasites. The data were analyzed and compared to the control group.

**Results**: The results of LC50 of glucantime and extract of *S. bavanati* against *Leishmania* (L) *tropica* were calculated; as expected, the LC50 of the static stage in the case of both compounds of the *S. bavanati* and glucantime in mg/mL was greater than the dynamic stage. Examination of the effect of extract of *S. bavanatia* on the survival of the promastigotes of *L. tropica* in concentrations of 0.2, 1, 5, 25, & 125 after 24, 48, and 72 hours using the repeat measure test revealed that extract of *S. bavanatia* did not show any significant difference after 24 and 48 hours.

**Conclusion**: S. *bavanati* extract did not show any significant effects on the *L. tropica* promastigotes in low concentrations, especially when the duration of exposure and the presence of the drug is short. On the contrary, when the concentration of the extract increases and the exposure time of the parasite.

Keywords: *Leishmania tropica*, in vitro, promastigotes, Correspondence Email(s): a.fattahi@ssu.ac.ir Poster

In vitro effect of linalool on RH strain of Toxoplasma gondii

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**Background**: Toxoplasmosis is a common zoonotic disease in humans. Recent studies aim to find more potent anti-*Toxoplasma* agents with less side effects. Linalool has shown considerable antioxidant and antimicrobial effects. In this study, we investigated the in vitro antiparasitic effects of linalool on *Toxoplasma gondii* (*T. gondii*) RH strain.

**Methods:** Linalool (97% purity, Sigma Aldrich, Germany) was dissolved in DMSO (0.06%). RH strain *T. gondii* tachyzoites were obtained from intraperitoneally infected BALB/c mice. In each tube,  $2 \times 105$  tachyzoites were exposed to different concentrations of linalool (37.5, 75, 150, 300, 600, 1200 µg/mL) and anti-parasitic effect was assessed using flow cytometry.

**Results**: linalool at concentration of 37.5, 150, 300, 600, and 1200  $\mu$ g/mL killed 18.37, 38.75, 47.57, 50.63, 74.44% of tachyzoites, respectively. In addition, the positive control, sulfadiazine, showed 67.85% lethality rate, while it was 11.25% in the negative control group (containing tachyzoites and propidium iodide).

**Conclusion**: We showed a dose-dependent in vitro lethal effect of linalool against the RH strain of *T*. *gondii*. Accordingly, linalool may be considered as a promising anti-*Toxoplasma* agent. Future studies should evaluate the cytotoxicity and in vivo efficacy and safety of the compound.

Keywords: Linalool, Toxoplasma gondii, in vitro

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In vitro effect of tropisetron on *Echinococcus granulosus* protoscoleces and expression of calcineurin phosphatase gene

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**Background**: Tropisetron is a selective receptor antagonist of serotonin 3 that is widely used to counteract chemotherapyinduced emesis. It has been shown that inhibits the phosphatase activity of calcineurin in the calcium-signaling pathway. This study evaluated the in vitro effect of tropisetron on *Echinococcus granulosus* protoscoleces and calcineurin gene expression.

**Methods:** Genotype G1 protoscoleces of E. granulosus was obtained from hepatic hydatid cysts of sheep. In vitro exposure to tropisetron at 250  $\mu$ M, 150  $\mu$ M and 50  $\mu$ M concentrations and in various periods performed. The viability of the protoscoleces was determined microscopically, as well as gene expression assessments of calcineurin subunits A, B as main genes and GAPDH as reference gene, at concentrations of 250  $\mu$ M and 50  $\mu$ M after 24 h. Real-time PCR was also performed, and mRNA expression was assessed.

**Results**: Protoscoleces showed gradual morphological deterioration of evagination, tegumental blebbing and hook shedding. The lethal effect of tropisetron at 50  $\mu$ M, 150  $\mu$ M and 250  $\mu$ M after 3 days were 1%, 25%, and 100%, respectively. Gene expression analysis revealed that the tropisetron, significantly at 50  $\mu$ M upregulated calcineurin subunit B, while at 250  $\mu$ M downregulated the gene.

**Conclusion**: Tropisetron has dose-dependent protoscolicidal effects. The dual effect of tropisetron on gene expression might reflect its function either as a serotonin antagonist which does not increase intracellular calcium or as an inhibitor of the calcineurin phosphatase enzyme which increases intracellular calcium levels.

Keywords: Tropisetron, calcineurin, *Echinococcus granulosus*, protoscoleces Correspondence Email(s): msh2006ir@yahoo.com Poster

In vitro efficacy of albendazole-loaded β-cyclodextrin against protoscoleces of *Echinococcus granulosus* sensu stricto

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**Background**: Cystic echinococcosis (CE) is a helminthic disease that is caused by the larval stage of the dog tapeworm *Echinococcus granulosus*. Albendazole (ABZ) is the first-line treatment for CE; however therapeutic failure of ABZ against CE occurs because of the low aqueous solubility and consequently its erratic bioavailability in plasma.

**Methods:** We evaluated the apoptotic effects of ABZloaded  $\beta$ -cyclodextrin (ABZ- $\beta$ -CD) against protoscoleces (PSCs) versus ABZ alone. After 15 h of exposure, Caspase-3 enzymatic activity was determined by fluorometric assay in PSCs treated with ABZ and ABZ- $\beta$ -CD groups. To assess the treatment efficacy of ABZ- $\beta$ -CD against PSCs, mRNA expression of Arginase (EgArg) and Thioredoxin peroxidase (EgTPx) were quantified by Real-time PCR.

**Results**: A significant scolicidal activity of ABZ was observed only at a concentration of 800 µg/mL (100% PSCs mortality rate after 4 days of exposure), while the 200 and 400 µg/mL ABZ reached 100% PSCs mortality rate after 9 sequential days. The 400 µg/mL ABZ- $\beta$ -CD had 100% scolicidal rate after 5 days of exposure. 400 µg/mL ABZ- $\beta$ -CD induced higher Caspase-3 activity than their controls, indicating a more potent apoptotic outcome on the PSCs. Also, we showed that the 400 µg/mL ABZ- $\beta$ -CD can downregulate the mRNA expression of EgArg and EgTPx, indicating more potent interference with growth and antioxidant properties of PSCs.

**Conclusion**: In the present study, a significant scolicidal rate, apoptosis intensity and treatment efficacy was observed in PSCs treated with 400  $\mu$ g/mL ABZ- $\beta$ -CD compared to ABZ alone. This provides new insights into the use of nanostructured  $\beta$ -CD carriers with ABZ as a promising candidate to improve the treatment of CE.

**Keywords**: Albendazole, apoptosis, arginase, thioredoxin peroxidase **Correspondence Email(s):** Adelespotin@gmail.com



In vitro evaluation of copper oxide and titanium dioxide on *Toxoplasma gondii* 

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**Background**: *Toxoplasma gondii*, the coccidian protozoan parasite with worldwide distribution, is the agent of toxoplasmosis. The disease is life threatening in con-genital form and in immunocompromised patients. A study was conducted to evaluate the in vitro effects of nanoTiO2 and nanoCuO colloid on tachyzoites of *T. gondii*, RH strain.

**Methods:** Various concentrations (10, 20, 50 ppm) of nanoTiO2 and CuO colloid were added to tachyzoites of *T. gondii*, RH strain and incubated for 30, 60, 90 and 120 minutes. Tachyzoites were tested for mortality with methylene blue dye and MTT assay, and the changes between control and exposed parasites were examined.

**Results**: For both nanocolloid with methylene blue dye test and MTT assay, the highest mortality rate occurred at 50 ppm concentrations and after 120 minutes of exposure. These nanoparticles killed significant numbers of tachyzoites at the highest concentration during the period of time they were in their vicinity.

**Conclusion**: The present study was carried out in 2024 and showed NanoTiO2 and NanoCuO colloid was effective on tachyzoites of T.*gondii* RH strains. These nano-particles kill *T. gondii* tachyzoites. Furthermore, these nano-particles have the ability to enter tachyzoites and kill them from within.

Keywords: T.gondii, Nano-CuO Nano-TiO2, tachyzoites Correspondence Email(s): Rezadabaghi97@gmail.com Poster

In vitro evaluation of the inhibitory effect of violacein pigment obtained from *Janthinobacterium lividum* on *Trichomonas vaginalis* 

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**Background**: *Trichomonas vaginalis* is one of the most common non-viral infections in the world. The common treatment used to control this disease is metronidazole, which is losing its effects due to the spread of drug resistance. Therefore, it is necessary to find alternative bioactive compounds.

**Methods:** In this study, which was conducted by an experimental intervention method, violacein pigment was first extracted from Jantinobacterium lividum IBRC-M10951 strain using methanol, and its relative purity was confirmed by thin layer chromatography (TLC) and infrared spectroscopy (FTIR). Then its anti-parasitic effects on *T. vaginalis* parasite and HDF cell line were investigated.

**Results**: Violacein pigment in concentrations of 2500 and 1250 µg/mL in 24 hours caused 100% inhibition of parasite growth. The IC50 value of this pigment against parasites in 48 hours of treatment was 7.497 µg/mL. Also, the CC50 of this pigment on the cell line was calculated as 84.38 µg/mL. According to the results, violacein is effective against *T. vaginalis* parasite and its toxicity on the cell line is 11 times compared to the parasite (SI = 11.25).

**Conclusion**: According to the inhibitory effect of violacein pigment on *Trichomonas* parasite, this substance can be proposed as a bioactive compound to be used in the treatment of parasitic infections. With additional research such as better purification of this pigment, use of other violacein pigment-producing bacteria.

**Keywords**: *Trichomonas vaginalis*, violacein, *Jantinobacterium lividum* 

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In vitro evaluation of the potent anti-leishmanial activity of *Ferula tabasensis* alone or in combination with Shark cartilage extract against the standard Iranian strain of *Leishmania major* (MRHO/IR/75/ER)

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**Background**: The present study aimed to explore the antileishmanial effects of ethyl acetate extract, methanol extract, and fractions 1-4 (F1-F4) of Ferula tabasensis, alone or in combination with shark cartilage extract (ShCE), on *L. major* in vitro.

**Methods:** In this study, ethyl acetate, methanol, and n-hexane extracts were extracted from the aerial roots of *F. tabasensis* by the maceration method. The silica gel column chromatography was used to separate n-hexane extracts at varying polarities (F1-F4 fractions). Subsequently, the effects of extracts and fractions against promastigotes were assessed by the parasite counting method microscopic inhibition test and MTT assay. Besides, their effects on the infected macrophage cells and the number of amastigotes were investigated. Cytotoxicity was evaluated in non-infected J774A.1 macrophage cells. Finally, apoptosis induction of promastigotes, including infected and non-infected macrophages, was evaluated.

**Results**: The results indicated the highly potent activity of F. tabasensis extracts and F1-F4 fractions, alone or in combination with ShCE, against *L. major* promastigotes and amastigotes in a dose-dependent manner (P 0.05). The F1 fraction and methanol extract showed markedly higher toxicity compared to the other extracts and fractions, with 50% inhibitory concentrations (IC50/72 h) of 2.4  $\pm$  0.29 and 2.9  $\pm$  0.55 µg/mL against promastigotes and 1.79  $\pm$  0.27 µg/mL and 1.39  $\pm$  0.27 µg/mL against amastigotes (*P* = 0.001). Moreover, they had a high selectivity index (SI) due to the low toxicity of macrophages (*P* = 0.0001).

**Conclusion**: The available drugs for the treatment of leishmaniasis are highly toxic and extremely expensive, with low efficiency; therefore, the development of effective therapeutic compounds is essential. In this study, the F1 fraction and methanol extract of *F*. *tabasensis* showed potent efficacy against *L. major*, associated with low toxicity and apoptosis.

Keywords: Ethyl, acetate, methanol extract, *Ferula* Correspondence Email(s): smolaie83@gmail.com

Poster

In vitro evaluation of Yazd honey effect on *Leishmania major* [MRHO/IR/75/ER] promastigotes

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**Background**: Leishmaniasis is a parasitic illness carried by the bite of some species of sand flies. The cause of the disease is protozoan parasites of the genus *Leishmania*. This study investigated In vitro evaluation of Yazd honey effect on *Leishmania major* [MRHO/IR/75/ER] promastigotes in stationary and logarithmic phases.

**Methods:** Multifloral honey from Yazd, Iran, harvested in 2016-2017, was studied using the *L. major* strain [MRHO/IR/75/ER] in BALB/c mice. Amastigotes were extracted from mouse spleens, converted to promastigotes in NNN medium, and monitored in culture using slide microscopy and ELISA. Parasites were placed in vials with honey-infused liquid medium at different concentrations (6.25, 12.5, 25, 50 µg/mL) and incubated for durations ranging from zero and continuing at 6, 12, 24, 48, 72, and 96 hours.

**Results**: The results of the counts taken were measured against those of the control group and showed that parasite load reduced with increasing Yazd honey concentrations (6.25 to  $50\mu g/mL$ ) and contact duration (6-96 hours).

**Conclusion**: The findings revealed that honey has the capacity to curb the growth and survival of parasites, positioning it as a potential subject for research in both animals and humans.

Keywords: Leishmania major, honey, viability Correspondence Email(s): afbafghi@gmail.com ith International & 12th National Congress of Parasitology and Parasitic Diseases of Iran Alborz University of Medical, Sciences, Karaj, Iran May 21 - 23, 2024

Poster

In vitro lethal effect of piperine nanocrystals on protoscolices of hydatid cyst

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**Background**: Hydatid cyst is a common zoonotic disease. In search for novel antiparasitic agents, piperine has shown considerable antimicrobial activities. Nanocrystals preserve the original purity of the drug while improving its bioavailability. In this in vitro study, we investigated the lethal effects of piperine nanocrystals (PNC) on hydatid cyst protosclolices.

**Methods:** Protoscolices were prepared from sheep hydatid cysts under sterile conditions. The viability of protoscolex was assessed by 0.1% eosin staining. PNC were synthesized and characterized. Protoscolices were exposed to concentrations of 125, 250, 500, 750  $\mu$ g/ml at 5, 10, 20, 30, 60 and 120 minutes. 0.9% saline 20% saline were used as negative and positive controls, respectively. Cytotoxicity was also evaluated using L929 fibroblast cells by MTT assay.

**Results**: Surface electron microscopy (SEM) confirmed the size and morphology of the nanoparticles. UV spectrophotometry showed a single peak at 340 nm, characterized by piperine. Dynamic light scattering (DLS) showed average size of 356 nm for nanoparticles. The highest PNC dose (750  $\mu$ g/mL) showed 80.6±0.58 and 87.67 ± 0.58% lethality rates after 60 and 120 minutes, respectively. Interestingly, even low doses of PNC (125  $\mu$ g/ml), showed significant lethality rates at 5 and 10 minutes (50.67 ± 1.16 and 59.00 ± 1.73%, respectively). In addition, no cytotoxicity was observed after 24 h MTT assay.

**Conclusion**: Given the higher drug bioavailability, PNC could be considered as a potential anti-protoscolex composition in hydatid cyst treatment. Further in vivo studies are recommended to uncover the therapeutic application of the PNC.

**Keywords**: Hydatid cyst, piperine, nanocrystal, nanoparticle **Correspondence Email(s):** Aref\_teimouri@yahoo.com Poster

In vitro lethal effect of piperine-loaded niosome nanoparticles on protoscolices of hydatid cyst

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**Background**: Hydatid cyst is a common zoonotic disease. Recntly, studies aim to find more potent therapeutic agents with less side effets. Niosome nanoparticles improve drug delivery and bioavailability. In this in vitro study, we investigated the lethal effect of piperine-loaded niosome (PNN) on protoscolexes of hydatid cyst.

**Methods:** Protoscolices were prepared from sheep hydatid cysts under sterile conditions. The viability of protoscolex was checked by 0.1% eosin staining. PNN were synthesized and characterized. Protoscolices were exposed to concentrations of 66, 132, 265, 400, 530  $\mu$ g/ml at 5, 10, 20, 30, 60 and 120 minutes. 0.9% saline 20% saline were used as negative and positive controls, respectively.

**Results**: Surface electron microscopy (SEM) confirmed the size and morphology of the nanoparticles. Dynamic light scattering (DLS) showed average size of 234.1 nm and zeta potential of -40 mv for nanoparticles. In a dose-dependent manner, PNN showed  $80.6 \pm 7.5\%$  and  $87.67 \pm 0.58\%$  lethality rates at 400 µg/mL concertation after 60 and 120 minutes, respectively.

**Conclusion**: Given the higher drug bioavailability, PNN could be considered as a potential anti-protoscolex composition in hydatid cyst treatment. Further in vivo studies are recommended to uncover the therapeutic application of the PNN.

Keywords: Hydatid cyst, piperine, niosome, nanoparticle Correspondence Email(s): Aref\_teimouri@yahoo.com



In vitro protoscolicidal effects of mangosteen (Garcinia mangostana) hydroalcoholic extract on protoscolices of Echinococcus granulosus

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**Background**: Cystic echinococcosis is a zoonotic disease caused by the larvae stage of Echinococcus granulosus. The treatment of choice for cystic echinococcosis is surgery. This study aimed to evaluate the efficacy of hydroalcoholic extract of mangosteen fruit peel against protoscolices of hydatid cyst in vitro.

**Methods:** Liver hydatid cysts were collected from slaughterhouse, and the cysts fluid containing live protoscoleces was aspirated aseptically and stored at 4° C until use. Various concentrations of hydroalcoholic extract of mangosteen fruit peel (10, 20, 40 and 80 mg/mL) were exposed to collected protoscoleces for 10, 20, 30, 60 and 120 minutes. In addition, 20% hypertonic sodium chloride and PBS were used as positive and negative controls, respectively. Viability of the protoscoleces was assessed using 0.1% eosin. All experiments were repeated at least three times.

**Results**: Our findings showed that the viability of the protoscoleces decreased significantly with increases in mangosteen hydroalcoholic extract concentrations (P = 0.001). The mortality rates of protoscoleces with exposure to concentrations of 40 and 80 mg/ml of mangosteen hydroalcoholic extract for 60 min were 67.33 and 85.33%, respectively. Mortality of the protoscoleces was 97.3% after 120 min of exposure to 80 mg/mL concentrations of mangosteen hydroalcoholic extract. The IC50 value obtained about 95.20 mg/mL for 10 min of mangosteen hydroalcoholic extract exposure.

**Conclusion**: The results of this study revealed the hydroalcoholic extract of mangosteen fruit peel has a dose-dependent and time-dependent protoscolicidal effect on the protoscolices of Echinococcus granulosus. Further experiments are needed for isolation of active fractions and identification of the active components of mangosteen hydroalcoholic extract.

Keywords: Protoscolicidal, mangosteen, *Echinococcus* granulosus, in vitro Correspondence Email(s): fmikaeili@yahoo.com Poster

In vitro study on four types of commercial lectins on Leishmania infantum, L. major and L. tropica with stage-specific binding and Leishmania species identification

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**Background**: We aimed to verify the susceptibility of *Leishmania infantum*, *L. major* and *L. tropica*, to commercial lectins in order to identify the three *Leishmania* species.

**Methods:** The degree of agglutination was determined both macroscopically and microscopically and was scored negative (-) to positive (from 1+ to 4+) based on their percentage of agglutination.

**Results**: Jacalin and UEA-1 were capable of agglutination of *L. infantum* isolates in both logarithmic and stationary phases at a concentration of 1000  $\mu$ g/mL (100%). *L. tropica* isolates showed agglutination with the lectin UEA-1 in both logarithmic and stationary phases (62.5% and 87.5%). *L. major* and *L. tropica* showed 75% agglutination with lectin Jacalin in both logarithmic and stationary phases. L. tropica isolates showed 25% agglutination with the lectin WGA in the logarithmic phase. *L. infantum*, *L. major* and *L. tropica* isolates showed 25, 12.5 and 37.5% agglutination in the stationary phases.

**Conclusion**: Despite the fact, that JCA and I-UEA lectins were not able to completely separate *L. infantum*, *L. major* and *L. tropica*. WGA lectin and PHA lectin can help in separating the species of *Leishmania* parasites.

Keywords: *Leishmania*, lectin, agglutination Correspondence Email(s): mohebali@tums.ac.ir

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Poster

In vivo and in vitro effects of essential oil and hydro-alcoholic extract of *Artemisia dracunculus* on *Toxoplasma gondii* Albe

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**Background**: Current chemotherapy of toxoplasmosis are often complicated and associated by severe side effects. The aim of this study was to evaluate effects of hydro-alcoholic extract and essence of Artemisia dracunculus on *Toxoplasma gondii* in vivo and in vitro conditions.

**Methods:** The extract was prepared due to Soxhlet method. The essense was extracted by Clevenger device and analyzed by Gas chromatography equipped with HP-5MS capillary column. Tachyzoites of RH strain were incubated for 1.5 hours in various concentrations (0.05-4  $\mu$ l/mL) and (0.156-10 mg/mL) of essence and hydro-alcoholic extract respectively. Then, they were stained by PI and analyzed by FACS. To evaluate the therapeutic quality, tachyzoites were inoculated into five mice from each group. Three doses of essence (0.25, o.5 and 1 mg/kg) and extract (780, 1560 and 3120 mg/kg) were daily administrated (orally) 24 hours after inoculation due 3 days continuously.

**Results**: The Gas chromatography could identify 74.04% of the copmponents; Monoterpene: 31.95% and Sesquitrerpene: 34.43%. The ID50 of the extract was 1.24 mg/ml whilst the quantity of oil essence was 0.43 µl/mL. Statistically, the longevity mean of mice which received 0.5 µl/mL of essence and all of groups administrated by different dose of the extract were more than the quantity of the control group (P < 0.05).

**Conclusion**: It seems that this species has effective compounds on the parasite, thus the differentiation of these compounds can assist for the drug production.

Keywords: Toxoplasma, Artemisia dracunculus, in vitro, Mice

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In vivo and in vivo activity of albendazole in comparison with Albendazole/albumin solid lipid nanoparticles against Echinococcus granulosus

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**Background**: The aim of this study was to evaluate the effects of solid lipid nanoparticles containing albendazole/albumin (albendazole/albumin SLN) in comparison with albendazole.

**Methods:** The *Echinococcus granulosus* protoscolices were incubated with serial concentrations of albendazole/albumin SLNs and albendazole alone at 37 °C for 60 minutes. To evaluate their gastrointestinal absorption, BALB/c mice were treated by oral gavage with 1000  $\mu$ g/mL albendazole/albumin SLNs and albendazole alone, and then their serum level of albendazole was determined. To compare their side effects, BALB/c mice were orally treated with albendazole/albumin SLNs and albendazole alone at a concentration of 1000  $\mu$ g/mL and then their hematological parameters, including RBC, WBC, Platelets, and hemoglobin and serum level of liver enzymes, including AST and ALT were analyzed.

**Results**: Albendazole/albumin SLNs were significantly more effective in killing protoscoleces than albendazole alone. We found that the gastrointestinal absorption of albendazole/albumin SLNs was more than albendazole alone. Also, it was declared that albendazole/albumin SLNs less affected hematological parameters (RBC, WBC, platelets, and hemoglobin) and liver enzymes than albendazole alone. Finally, the viability percentage of protoscoleces was measured.

**Conclusion**: Taken together, it is found that the biological properties of albendazole are improved with the nano-based formulation. However, more in-vitro and in-vivo studies are needed for predicting formulations and molecular aspects of drugs for elimination of the parasitic diseases.

Keywords: Albendazole, albumin, solid lipid nanoparticles

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Insilico mining of microRNAs of *Blastocystis* sp. in irritable bowel syndrome (IBS) patients

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**Background**: MicroRNAs (miRNAs) are small regulatory RNA molecules that play a role in controlling gene expression by inhibiting translation and destabilizing target mRNAs. Research has shown that miRNAs are present and actively studied in various parasites, indicating their potential significance in the regulation of gene expression in these organisms.

**Methods:** To perform a bioinformatics investigation into human miRNAs associated with gastrointestinal diseases, a set of impactful miRNAs was assembled. The sequences of these miRNAs were acquired from https://www.mirbase.org/ and subsequently subjected to a similarity search using https://blast.ncbi.nlm.nih.gov to assess their similarity to *Blastocystis* mRNA. Sequences displaying a high degree of similarity to the *Blastocystis* mRNA genome were then selected for further analysis of their role in miRNA folding. After these assessments, the most promising miRNA was chosen for primer design and synthesis to enable in vitro experiments.

**Results**: Out of the 150 miRNAs analyzed, miRNA 29a-5p was found to have an identical initial 14 nucleotides to *Blastocystis* mRNA in subtype 1. According to the miRNA fold site, it was determined that miRNA 29a-5p could potentially function as a miRNA in *Blastocystis* sp.

**Conclusion**: The investigation of miRNAs is essential due to their involvement in various diseases and their regulatory influence on gene expression. Although miRNAs have been extensively studied in several parasites, their presence in *Blastocystis* sp. has not been definitively confirmed. The identified miRNA sequence within the *Blastocystis* genome has been suggested.

Keywords: *Blastocystis* sp, microRNA, IBS, miRNA29a-5p Correspondence Email(s): majid\_pirestani@yahoo.com Poster

Intermediate host of *Fasciola hepatica* from northeast to south of Iran: *Galba truncatula* or *Galba schirazensis*?

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**Background**: Snails of the Lymnaeidae family are the intermediate hosts of the *Fasciola hepatica* infection in the Middle East. Although *G. truncatula* is known as the main intermediate host in Iran but in recent years, there have been conflicting reports of the ability of G. schirazensis to host *F. hepatica*.

**Methods:** Snail specimens were collected from 42 water bodies of Kerman, Fars, Razavi and North Khorasan Provinces. Each specimen were identified using conchological characteristics as well as PCR-sequencing and phylogenetic analyses of ITS2 and Cox1 genes. A *Fasciola*-specific PCR amplification of noncoding tandem repeat gene fragment of 124 bp were performed for identification of the snail infection.

**Results:** *G. schirazensis* and *G. truncatula* were isolated from 4 and 38 regions respectively. Molecular studies on the collected samples did not reveal any evidence of natural Fasciola infection in *G. schirazensis*. *G. truncatula* isolates collected from 5 out 38 habitats were infected with *F. hepatica*.

**Conclusion**: Findings of this study indicated *G. truncatula* as the main intermediate host of *F. hepatica* in most of the parts of Iran, similar to that of Guilan province. In this study the two species were not found in the same location and our knowledge on the distribution of *G. schirazensis*.

Keywords: Fasciola hepatica, Galba, Iran

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Intestinal parasites among patients referred to ImamAli hospital of Sarayan, south Khorasan province in Iran from 2019 to 2022

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**Background**: The diseases caused by intestinal parasites are considered as one of the most important public health problems. In this study, we assessed the frequency of intestinal parasites in patients referred to Imam Ali Hospital, South Khorasan province, Iran from 2019 to 2022.

**Methods:** : In this study, we evaluated 2072 patient files referred to Imam Ali Hospital in South Khorasan Province, Iran regarding intestinal parasites including protozoa and helminth from 2019 to 2022. All the fecal samples were investigated by direct wet mount technique using saline and Lugol's iodine solution. All the demographic information such as sex and age were collected alongside with hospitalization, year, season, and time of detection. The age group was considered as 0-2, 2-6, 6-10, 10-20, and 20.

**Results**: We reported *Giardia duodenalis*, *Enterobius vermicularis*, *Entamoeba histolytic*, *Entamoeba coli*, *Trichomonas hominis*, *Chilomastix mesnili*, and *Blastocystis hominis*. The highest protozoon in spring was *E. coli* (44.4%) followed by *G. duodenalis* (33.3%). *G. duodenalis* was the most reported parasite in summer and autumn with 46.2%, 38.5%, respectively. In winter, the highest intestinal parasites was regarding to *G. duodenalis* (33.3%). The highest infection was reported in the age group of 2-6 years old with *G. duodenalis* (37.5%). Among both hospitalized and out patients, *E. coli* (33.3%) followed by *G. duodenalis* (25%) were the highest ones.

**Conclusion**: The results of this study may contribute to design strategies to control of the intestinal parasites in this area.

Keywords: Intestinal parasitic disease, south Khorasan

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Poster

Intestinal parasitic pathogens among malignant patients in Zanjan province, northwest of Iran

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**Background**: Intestinal parasitic infections are major concern in immunocompromised patients particularly in developing countries. The present study was carried out to evaluate the frequency of intestinal parasites in malignant patients in zanjan province, northwest Iran.

**Methods:** Fecal specimens were collected, in this cross-sectional study, from 150 malignant patients and were examined using wet-mount, formalin-ethyl acetate concentration, trichorome and modified Ziehl-Neelsen staining and agar plate culture.

**Results**: The total infections rates were 6% (9/150). *Blastocystis* hominis was the most predominant parasite flowed by *Giardia lamblia* and *Chilomastix mesnilli*.

**Conclusion**: This was the first report on the presence of intestinal parasites in cancer patients in zanjan province. The findings emphasize that special consideration should be paid to immunocompromised individuals in order to prevent the infections associated with intestinal parasites.

**Keywords**: Intestinal parasites, malignancy, Zanjan, Iran **Correspondence Email(s):** apezeshki@zums.ac.ir



Introducing three medical species of solpugids for the first time in central Iran

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**Background**: Solpugids do not have poison glands or sting, but they can hunt animals larger than themselves using their powerful and large chelicerae. Solpugids contain one thousand known species and 153 genera. Given the importance of solpugids in terms of causing fear, discomfort and the possibility of infection in case of

**Methods:** Sixty five specimens of solpugids collected from homes and dormitories in Kashan City by hand-collecting method and were transferred to laboratory. The collected solpugids were preserved in 70% ethanol and were identified using special and valid keys under stereomicroscope in the laboratory.

**Results**: The results revealed that at least 3 species of solpugids from Rhagodidae, Galeodidae and Gylipidae families exist in Kashan city. Out of 65 specimens collected, 51% were from Galeodes caspius species , 41% Gylippus lamelliger and 7.5% Rhagodes melanochaetus. This study only consisted of solpugids that had left their main location and habitat or place of birth to feed or do other activities. All specimens were collected after being observed or entering the residential houses or around the houses. Meanwhile, all specimens were collected from March to October. The highest abundance belonged to months from June to August.

**Conclusion**: Lack of awareness about the life of this animal has caused an unfounded fear of it that since the distant past has caused fear and anxiety in people. Due to having powerful chelicerae, in case of biting they may cause deep wounds that become infected. Through training the biological characteristics

Keywords: Camel spiders, solpugid, fauna

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Poster

Investigating the effect of parasites (Toxoplasma, *Leishmania*, and Hydatid cyst) antigens on Alzheimer's animal model

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**Background**: Alzheimer's is a severe disorder that affects memory, cognition, and behavior. Some studies have suggested that exposure to certain infectious agents in early life may offer protection against Alzheimer's. This study aims to investigate the effects of parasite antigens on Alzheimer's symptoms in an animal model.

**Methods:** Alzheimer's model was induced in Wistar rats using Amyloid beta-peptide (A $\beta$ ), the animals were treated with crude antigens from *Toxoplasma*, *Leishmania*, and hydatid cysts. Spectrophotometry and real-time were used to evaluate, antioxidant enzyme activity, and gene expression of NLRP3, IL-8, IL-1 $\beta$ , and Caspase-1. Histological assay were also conducted to investigate structural changes in the hippocampus and beta amyloid deposition in the hippocampus and cortex. Apoptosis was analyzed by Flow cytometer.

**Results**: The state of total oxidant, antioxidant and SOD increased in the Alzheimer's group compared to the control group, but the amount of these factors was lower in the group treated with *Leishmania* antigen compared to other treatment groups. The apoptosis in the treated groups was lower compared to the Alzheimer's group, but there was no significant difference. IL-8 expression was significantly higher in all Alzheimer's groups compared to the control group, but decreased in those treated with hydatid cyst and *Leishmania* antigens compared to Alzheimer's. IL-1 $\beta$  and Caspase-1 expression were similarly increased in all groups compared to the control group,

**Conclusion**: The findings suggest that parasite antigens, specifically *Leishmania* antigens, may have neuroprotective effects that reduce oxidative stress, apoptosis, inflammation, and histopathological changes in response to Alzheimer's in an animal model.

Keywords: Alzheimer, Toxoplasma, Leishmania, hydatid cyst

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Investigating the effects of cat contact and red meat consumption on *Toxoplasma gondii* infection in Mashhad pet clinic employees

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**Background**: *Toxoplasma gondii* is a common protozoan that infects warm-blooded animals. It is found wherever cats are present and can be transmitted to humans. Veterinary clinic workers are at higher risk of infection due to their close contact with cats.

**Methods:** The collection of 92 blood samples was carried out in the pet clinics in Mashhad City through blood collection from employees. These people were also asked about their daily contact with different cats and the frequency of red meat in their weekly diet. The blood was taken to the laboratory in clotted tubes and their serum was separated. An anti-*Toxoplasma gondii* IgG antibody assay kit was used to measure this antibody in the serum of these people by ELISA method.

**Results**: The prevalence of IgG in this occupational group was 13.4%. By performing the *Chi-square* statistical test, the p-value of daily contact with cats was 0.007 and the amount of weekly consumption of red meat in the food plan was 0.077. This study suggests that employment in small animal clinics does not pose a significant risk of contracting *Toxoplasma*.

**Conclusion**: The prevalence rate in these workers is not significantly different from other communities in Mashhad. these people should be fully aware of the parasite cycle and should take the necessary preventive and hygienic measures when interacting with cats and preparing and cooking meat to prevent themselves from contracting this parasite.

**Keywords**: *Toxoplasma gondii*, Mashhad, seroprevalence **Correspondence Email(s):** abolfazlalizadeh304@gmail.com Poster

Investigating the efficacy of quinolone-coumarin hybrids against *Toxoplasma gondii* using in vitro

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**Background**: Toxoplasmosis, caused by *Toxoplasma gondii*, poses global health concerns, particularly for vulnerable populations. Consequently, there is a need for new drugs that cause minimal damage to host cells. The purpose of this study was to investigate the antiparasitic efficacy of quinolone–coumarin hybrids QC1–QC12 against *T. gondii*.

**Methods:** The derivatives were compared with novobiocin and ciprofloxacin during testing, with pyrimethamine used as a positive control. We conducted the MTT assay to examine the anti-toxoplasmic effects of the test compounds and novobiocin. Evaluation included the infection and proliferation indices, as well as the size and number of plaques (areas with lysed cells), based on the viability of both healthy and infected cells. Additionally, we studied the behavior of tachyzoites after treatment with quinolones and novobiocin.

**Results**: The in vitro assays revealed that QC1, QC3, QC6, and novobiocin, with selectivity indices (SIs) of 7.27, 13.43, and 8.23, respectively, had the least toxic effect on healthy cells and the highest effect on infected cells compared to pyrimethamine (SI = 3.05). Compared to pyrimethamine, QC1, QC3, QC6, and novobiocin without having a significant effect on cell viability, demonstrated a significant effect on reducing in both infection index and proliferation index, in addition to reducing the quantity and dimensions of plaques (P < 0.05).

**Conclusion**: Based on our results, QC1, QC3, QC6, and novobiocin due to their significant therapeutic effects could be considered as potential new leads in the development of novel anti-*Toxoplasma* agents.

Keywords: Toxoplasmosis, quinolones, coumarins, novobiocin, antiparasitic

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## Investigating the interaction of chloroquine with wild and mutated forms of pvcrt-o and pvmdr-1 proteins of *Plasmodium vivax* via molecular docking and simulation

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**Background**: Malaria remain a major public health concern worldwide in which *Plasmodium vivax* a subtype of malaria parasite, generate the second outmost causes of malaria in the world due to its low mortality and its geographical distribution among African-Asian countries. Chloroquine (CQ) is the prior medication for anti-*P. vivax* therapy. Since

**Methods:** Molecular docking used to find the binding pose and binding affinity of CQ in PVMDR1/PVCRT-0 wild and mutant protein's active site using Autodock 4.2.2. The lowest bnding energy selected for further investigation. MD simulation were performed to study the molecular bases of CQ with PVMDR1/PVCRT-0 wild and mutant protein, applying AMBER99SB force field in GROMACS 2019.6 program.

**Results**: Molecular docking result indicated the suitable binding pose and binding energy of CQ with wild type PVMDR1/PVCRT-0 compared to its mutant allele. The binding energies of wild PVMDR1 (-7.55kcal/mol) and wild PVCRT-0 (-7.20) with CQ as in comparison to mutant PVMDR-1(-6.65kcal/mol) and mutant PVCRT-0 (-6.70) with CQ, represent the wild type proteins of PVMDR-1/PVCRT-0 has lowest binding energy. The MD Simulation of confirms the docking result through analysis of RMSD, RMSF, SASA, RG and so on.

**Conclusion**: The outcome of present study reinforce that the mutations here investigated on PVMDR1/PVCRT-0 could be potential good markers of *P. vivax* chemoresistance to CQ.

Keywords: PVMDR-1, PVCRT-0, P. vivax, bioinformatics

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Investigating the percentage of *Ascaris lumbricoides* transmission from infected dogs to their owners during one year in two veterinary hospitals in the west of Mazandaran province

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**Background**: Considering that, the Ascaris lumbricoides is one of the common parasites between humans and canines. In this research, we examined the rate of infection of the owners of dogs with this parasite. According to the results, about twenty-five percent of the owners of infected dogs were also infected with parasite.

**Methods:** The results of this research obtained during one year (from April 2020 to April 2021) from two veterinary hospitals in the west of Mazandaran province. First, stool samples were taken from suspected dogs. Then we selected dogs whose stool samples were positive in the next step we included the owners of these dogs in our statistical population. We asked the owners of the affected dogs to take tests if symptoms appear and inform us of the test results.

**Results**: According to the results, about twenty-five percent of the owners of infected dogs were also infected with this paradite. Of this percentage, sixty-seven percent were the owners of dogs that were kept outside the house and were younger.

**Conclusion**: Considering that, most of the infected with this parasite were the owners of dogs that were kept outside and were younger, parasite therapy and compliance with health principles in dealing with dogs with this condition should be the priority of dog owners and their families.

**Keywords**: *Ascaris lumbricoides*, zoonosis, canine parasite

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Investigating the prevalence of *Blastocystis* spp and Subtype analysis of *Blastocystis* isolates using SSU rRNA sequencing in patients with HIV-positive in Urmia, northwestern Iran

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**Background**: *Blastocystis* spp is a common and opportunistic protozoan that is found in individuals with compromised immune systems, such as those with HIV, compared to healthy individuals. Based on this, we investigated the prevalence of *Blastocystis* and Subtype analysis of *Blastocystis* isolates using Small Subunit Ribosomal RNA Genes (SSU).

**Methods:** In this cross-sectional study, 70 stool samples were collected from patients who were HIV-positive. The samples were examined using the wet slide method (physiology and Lugel's serum) and formalin ether test for the presence of *Blastocystis* spp and were cultured in Jones' special medium and positive samples were isolated. After extracting DNA from positive samples, the samples were analyzed by the Polymerase Chain Reaction (PCR) method. Finally, the subtypes were determined by the SSU rRNA sequencing and PCR methods. In addition, phylogenetic analyzes and genetic diversity were also performed.

**Results**: In the preliminary investigations, 25 (35.7%) of the 70 samples, were positive by direct microscopic method and formalin ether sedimentation method. also, Out of 70 samples in Jones' special culture medium, 25 (35.7%) positive samples were obtained. Out of 25 positive samples identified, only 23 samples (32.8%) created the band related to SSU rRNA gene fragment. Sequencing showed that 12 (52.2%), 7 (30.4%), and 4 (17.4%) isolates are related to genotype III, II, and I, respectively. Genotype IV was not isolated. In addition, there was a significant relationship between different parasite subtypes with TCD4 and gastrointestinal symptoms (P = 0.001).

**Conclusion**: The results of our study indicate that *Blastocystis* is quite common among individuals with HIV. This may be a consequence of the diminished TCD4 cell count observed in these patients. Nevertheless, additional research is needed to explore this topic further.

Keywords: *Blastocystis hominis*, HIV, SSU rRNA Correspondence Email(s): arashaminpour@gmail.com Poster

Investigating the prevalence of sarcocystosis and liver flukes in animals at Hamedan Industrial Slaughterhouse, 2023

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**Background**: *Sarcocystis* species as obligate heteroxenous coccidia, infections in the form of cysts in muscle tissue in livestock. *Fasciola* and *Dicrocoelium* species are liver trematodes that infect mammals and humans. This study was carried out to determine the prevalence of sarcocystosis, and liver Flukes infection in slaughtered animals at Hamadan slaughterhouse.

**Methods:** In this study, 3067 sheep carcasses (74%), 580 goat carcasses (14%), and 497 cow carcasses (12%) were examined. The livers of the animals were examined for appearance and the presence of liver trematodes. Additionally, their muscles were examined macroscopically for the presence of *Sarcocystis* species.

**Results**: In sheep, 86 livers were infected with *Dicrocoelium dendriticum* (2.8%), 10 livers were infected with *Fasciola hepatica* (0.32%) and 12 carcasses were infected with *Sarcocystis* species (0.4%). In goats, 3 livers were infected with *Dicrocoelium* (0.52%), 1 liver sample was infected with *Fasciola* (0.17%) and *Sarcocystis* (0.69%) was detected in 4 carcasses. In cows, 2 livers were infected with *Dicrocoelium* (0.4%), 1 was infected with *Fasciola* (0.2%), and 18 carcasses were infected with *Sarcocystis* (3.6%).

**Conclusion**: Based on the results obtained, although the prevalence of sarcocystosis and liver trematode infection in the studied animals was not significant, health measures are necessary to control and reduce these infections in livestocks due to their health and economic importance.

**Keywords**: *D. dendriticum*, *F. hepatica*, *Sarcocystis*, livestocks, slaughterhouse

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Investigating the rate of transplacental infection of *Neospora caninum* from cattle to apparently healthy fetuses in Kermanshah slaughterhouse

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**Background**: Neosporosis is one of the most important livestock diseases with global prevalence, which mainly causes abortion and infertility and causes great damage to the livestock industry.

**Methods:** 194 blood samples including 65 pregnant cows and 65 of their fetuses, as well as 64 non-pregnant cows were taken from Kermanshah slaughterhouse. The samples were tested using PCR method.

**Results**: *Neospora caninum* genome was observed in the blood of 30 pregnant cows (46.15%) and 9 out of 65 fetuses (13.84%). If the presence of the genome in the blood could considered as active parasitaemia, the mothers of 8 infected fetuses showed parasitaemia, while one infected fetus was obtained from a cattle without parasitaemia. The prevalence of vertical transmission in cows with and without parasitaemia was 8 of 30 (26.67%) and 1 of 35 (2.85%), respectively. The odds ratio of vertical transmission was calculated to be 12.36 (P-value 0.001). Eight of 9 infected fetuses were less than 120 days old.

**Conclusion**: Probability of vertical transmission in cows with the parasite's genome in their blood is much higher than other ones. The probability of infection at the early embryonic stages is much more.

Keywords: Neospora caninum, cattle, vertical, fetuses

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Poster

# Investigating the relationship between age and type of blood group with positivity of *Toxoplasma gondii* serum IgG in the employees of Mashhad small animal veterinary clinics

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**Background**: Toxoplasmosis is caused by the parasite *Toxoplasma gondii*, which affects warm-blooded animals and humans. Because it has been proven in various studies that blood groups may affect the severity and incidence of infectious diseases. This study examines the relationship between blood group, age and toxoplasmosis in humans.

**Methods:** 92 blood samples were collected by employees from pet clinics in Mashhad city. Blood is transported to the laboratory with a sealed tube and after the blood type test, blood serum was separated. Then, by using commercial ELISA kits to test antibodies against *T. gondii*, the infection of people with this parasite was investigated.

**Results**: In this community, none of the people under 25 years of age tested positive. 10.7% of people under 30 years of age, 16.7% of people under 40 years of age, and 50% of people over 40 years of age had a positive test result by performing the *Chi-square* p-valve statistical test, this variable was obtained with a positive test. However, in the investigation of the relationship between the blood group and the positive result of the test, no significant relationship was found.

**Conclusion**: The results of this study support the relationship between *Toxoplasma* infection and age. As people, get older and more likely to be exposed to parasites, especially because of the jobs these people have, the prevalence of serum antibodies against *Toxoplasma* increases with age.

Keywords: Toxoplasma gondii, age, blood group

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Investigating the relationship between pathogenic bacteria and Acanthamoeba in natural water resources

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**Background**: Acanthamoeba is the cause of granulomatous encephalitis. Helicobacter pylori, Campylobacter jejuni, and Acinetobacter baumannii cause adenocarcinoma, gastroenteritis, and endocarditis, respectively. pathogens reproduce inside amoeba, which causes death, contamination of new parasites and disease. The purpose of study was to identity of endosymbionts and Acanthamoeba in the hot water of Arak.

**Methods:** In this cross-sectional study, 46 samples of fixed and floating biofilms were collected in men's and women's indoor swimming pools, small ponds, tubs, reservoirs, spa faucets, springs, and plumbing of local spa springs. After the microscopic and molecular diagnosis of Acanthamoeba, the identity of endosymbionts of *Acinetobacter baumannii*, *Helicobacter pylori* and *Campylobacter jejuni* in *Acanthamoeba* positive samples was evaluated by Mutiplex-PCR method.

**Results**: In total, out of 46 samples, 31 samples had positive microscopic results and 11 Acanthamoeba isolates (35.5%) were found to be molecularly positive in hot springs. Out of 11 PCR positive isolates of Acanthamoeba (genotype T2 and Protoacantabohemica), 3 isolates (27.3%), *Helicobacter pylori* 0 isolates (0%) and *Campylobacter jejuni* 5 isolates (45.5%) were found to be infected with *Acinetobacter baumannii* according to type and sampling units. 9.09% floating biofilm and 27.27% of 11 PCR-positive *Acanthamoeba* isolates were infected with *Acinetobacter baumannii* and *Campylobacter jejuni*, respectively, and 8.18% of fixed biofilms were infected with each of *Acinetobacter baumannii* and *Campylobacter jejuni*.

**Conclusion**: This study showed that *Acinetobacter baumannii*, *Helicobacter pylori* and *Campylobacter jejuni* have the ability to infect *Acanthamoeba* in natural hot spring water conditions. The presence of free-living amoebae along with pathogenic bacteria (endosymbionts) in hot springs can be considered a serious threat to the health of people using these springs.

Keywords: Acanthamoeba, A. baumannii, H. pylori, C. jejuni, endosymbiont. Correspondence Email(s): dalimi\_a@modares.ac.ir Poster

Investigating the seroprevalence of *Toxocara canis* and *Toxoplasma gondii* parasites in people with multiple sclerosis: a cross-sectional study

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**Background**: Multiple sclerosis (MS) is an autoimmune complication whose cause is not fully known and hypotheses have been proposed about the possible influence of infectious agents such as parasites on the causes or progress of the disease.

**Methods:** Objective: Investigating the seroepidemiology of *Toxocara canis* and *Toxoplasma gondii* among patients with MS and the control group and related epidemiological and demographic factors. Blood samples from eighty MS patients and eighty healthy individuals were analyzed for the anti-*Toxocara* and anti-*Toxoplasma* antibodies (IgG) levels that were checked using the ELISA method.

**Results**: The results showed that the prevalence of serum antibody against *T. canis* in the patient group is higher than the healthy group (control) (42.5% vs. 35%). In contrast, the serum prevalence of anti-*T. gondii* antibody was lower in the control group (58.75% vs. 68.75%). In terms of age, education and gender, there was no significant difference between patients with multiple sclerosis and the control group (P > 0.05).

**Conclusion**: This study shows that, despite the lack of statistical association between parasite prevalence and MS, their high prevalence in this group should be considered.

Keywords: *Toxocara canis*, *Toxoplasma gondii*, Multiple sclerosis Correspondence Email(s): zibaeim@sums.ac.ir



Investigating the therapeutic effects of curcumin nanocapsules in hydatid cyst-infected mice

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**Background**: The present study aims to provide insights into the effectiveness of curcumin nanocapsules in treating hydatid infections.

**Methods:** Following five months after infection, 45 mice were divided into six groups. Groups 1, 2, and 3 were treated daily with curcumin nanocapsules (0.5, 0.25, 0.125 mg/mL) for one month. Group 4 was treated with curcumin (0.5 mg/mL), group 5 was treated with albendazole (150 mg/kg), and group 6 was the negative control group without treatments (only received saline).

**Results**: A detailed analysis of the cysts' physical characteristics, including their size and weight, has been conducted, and the data obtained has been recorded in designated tables. Regarding the total cyst numbers, all three nanocapsule groups had significantly lower total cyst numbers than the curcumin, albendazole, and negative control groups. Regarding the total cyst weight, all three nanocapsule groups had a significantly lower total cyst weight than the curcumin and negative control groups. Regarding the total cyst weight, all three nanocapsule groups had a significantly lower total cyst weight than the curcumin and negative control groups. Regarding the cyst with the maximum size, nanocapsules groups 1 and 2 had a significantly smaller size than the curcumin, albendazole, and negative control groups.

**Conclusion**: In conclusion, curcumin nanocapsules can serve as suitable alternatives to chemical drugs.

**Keywords**: Hydatid cyst, curcumin, nanocapsules

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Poster

Investigation of a new mutation in LmAQP1 from Leishmania major and molecular characterization of aquaglyceroporin (MRHO/IR/75/ER)

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**Background**: Pentavalent antimony such as sodium stibogluconate and meglumine antimony are considered the first line of treatment for cutaneous leishmaniasis. One of the most important ways of drug absorption is by a membrane protein called aquaglyceroporin coded by Aquaglyceroprotein1 (LmAQP1). In this study, the molecular characteristics of LmAQP1 were reported.

**Methods:** Cell culture of promastigotes of *Leishmania major* (MRHO/IR/75/ER) was performed and then DNA extraction, RNA extraction, and cDNA synthesis were performed. The amplicons obtained from PCR and RT-PCR were sequenced. Molecular characterization was performed by bioinformatics software such as BLST, ClustalW2, and RMSD.

**Results**: The amplicons obtained from PCR and RT-PCR showed the same size in terms of length. BLASTn analysis revealed a single point nucleotide change in the LmAQP1 gene, which encodes a 282 amino acid long protein, with a mutation at position 154 including an alanine to threonine substitution. The mutation observed in the desired gene was analyzed using the above software. The mentioned gene was deposited in GenBank, NCBI under accession number KU514052.

**Conclusion**: The function of the encoded protein of LmAQP1 showed that the said mutation could not affect the three-dimensional structure, but might change the drug uptake potential of this important channel. Based on the role of LmAQP1, it seems to be a good candidate for drug development.

**Keywords**: Aquaporin 1, *Leishmania*, molecular characterization

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Investigation of demographic, parasitology and geographical distribution characteristics of patients with amebiasis and shigellosis from 2012 to 2023 in Health Centers of Mazandaran University of Medical Sciences

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**Background**: The causative infectious diarrhea in developing countries are mostly bacterial pathogens while parasites account for less than 5% of cases of diarrhea. This study aims to investigate the demographic and clinical characteristics of patients with amebiasis and shigellosis in the population covered by Mazandaran University of Medical Sciences.

**Methods:** All confirmed cases of amebiasis and shigellosis in Mazandaran province between 2012 and 2023 were included in the study. To collect the required data, we used the registered information from the health networks and health department of Mazandaran province as well as the website of the Infectious Diseases Management Center of the Ministry of Health. The obtained data were analyzed using SPSS statistical software version 23.

**Results**: A total of 2,346 cases of bloody diarrhea were reported during the study period, of which 88 cases grew Shigella in stool culture and 339 cases showed Entamoeba histolytica cysts or trophozoites. The highest prevalence for Shigella and ameba was observed in the under 10 years age group, with 10.01 and 27.10 per hundred thousand people, respectively. The most cases of Shigella were seen in Ramsar and the most cases of ameba in Nowshahr.For ameba, the most common year and month were 2019 (85 cases, 25.07%) and December (46 cases, 13.56%), respectively.

**Conclusion**: The results show that the prevalence of amebiasis in Mazandaran province increased significantly in the last year of the study period compared to previous years, highlighting the need for preventive measures. Shigellosis and amebiasis were found in both genders almost equally, with a slight preference for males.

**Keywords**: Amebiasis, shigellosis, infectious diarrhea, epidemiology

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Poster

Investigation of *Leishmania* infection in sandflies vector and rodents in west of Iran

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**Background**: Cutaneous leishmaniasis is a prevalent tropical and subtropical disease transmitted by the bite of Phlebotominae sandflies. This disease remains a public health concern in Iran; therefore, the aim of present study was to examine *Leishmania* infection in sandflies and reservoir rodents in rural regions of Nahavand county, western Iran.

**Methods:** During May to October 2022, sandflies and rodents specimen were collected in six rural areas of Nahavand County. Wire traps and sticky traps were used to collect the specimens. The collected specimens were identified using valid diagnostic keys up to species level. Female sandflies (n = 289) and lesion/earlobe specimens from rodents (n = 61) were used for *Leishmania* DNA detection by ITS2 and 18S rRNA PCR and positives were Sanger sequenced.

**Results**: 3396 sandflies including, *Phlebotomus papatasi* (42.7%), *P. major* (20.6%), *P. mascitti* (0.27%), *P. neglectus* (0.21%), *P. alexandri* (0.24%), *P. turanicus* (0.35%), *Sergentomyia sintoni* (18.1%), *S. dentata* (10.5%), *S. theodori* (5.83%), *S. antennata* (1.09%), and *S. pawlowski* (0.09%) were caught. Two *L. major* infections in *P. papatasi* and one *Leishmania* sp. in *P. major* observed. This is the first report of *Leishmania* infection in sandflies from Hamadan province. Furthermore, 61 rodents including, *Arvicola amphibius* (37.7%), *Mus musculus* (29.5%), *Microtus socialis* (13.11%), *Apodemus sylvaticus* (11.47%), *Talpa davidiana* (4.9%), *Apodemus witherbyi* (1.63%), and *Rattus norvegicus* (1.63%) were captured; which molecular examination scored negative.

**Conclusion**: The presence of *Leishmania* infection in the Phlebotominae sandflies in Nahavand, indicate a potential threat to humans and animals in the region. Regular monitoring and examination of the sandflies population, as well as timely diagnosis and treatment of new patients, are strongly recommended.

Keywords: Leishmaniasis, *Leishmania major*, sandflies, rodent Correspondence Email(s): a.maghsood789212@gmail.com



Investigation of *Leishmania* infection in stray dogs from Kermanshah

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**Background**: One of the important diseases in Canidae family is leishmaniosis which threat their health and causes muscle weakness and lethargy. This disease is considered as a zoonotic disease, which is presented in 88 developing countries. The majority of the infected dogs do not show signs.

**Methods:** The aim of this study is evaluating the seroprevalence of *Leishmania infantum* among stray dogs in Kermanshah city. Ninety-two stray dogs, 30 males and 62 females, were randomly selected and 5 mL blood was drawn. The blood was analyzed by two serologic tests. Serum samples were evaluated for the presence of antibodies using Enzyme-linked immunosorbent assay. Next, positive samples were investigated with direct agglutination test (DAT).

**Results**: From 92 blood samples which were analyzed by ELISA, 11 samples (11.95%) were positive. The antibody titration of positive samples was assessed by DAT. There was no statistically significant difference between genders.

**Conclusion**: The high frequency of infection among examined dogs indicates that the stray dogs can probably be regarded as the principal source of infection for individuals living in this area. We recommend frequent surveillance of leishmaniasis in dogs especially stray dogs in order to decrease disease incidence in humans.

Keywords: *Leishmania* infection, ELISA, canidae, Kermanshah Correspondence Email(s): shokrani.hm@lu.ac.ir Poster

Investigation of parasitic contamination and their molecular characteristics in oil refinery wastewater

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**Background**: Parasites are typically transmitted through several routes, such as direct contact with infected persons or animals, and ingestion of infective cyst/oocyst/spore through contaminated water or food. The ORW contains toxic materials, and a few microorganisms can stay active in these environments.

**Methods:** Samples were collected from an ORW facility for nine months. Samples were either filtered using 0.4  $\mu$ m cellulose nitrate membrane or sedimented. DNA extraction was performed for all samples. The presence of targeted parasites was evaluated using real-time PCR. Genotyping/subtyping of positive samples was characterized using sequencing.

**Results**: Real-time PCR showed that from 76 ORW samples, targeted protozoa were identified in 26 (34.2%) samples. *Blastocystis* sp., *Enterocytozoon bieneusi*, and *Giardia duodenalis* were detected from 9.21% (7/76), 25.0% (19/76), and 2.63% (2/76) of samples, respectively. *E. bieneusi* was the most common species detected in samples followed by *Blastocystis* sp. and *G. duodenalis*. *E. bieneusi* genotypes were BEB6 (3/5; 60%), C (1/5; 20%), and an unknown genotype. *Blastocystis* sp., ST1 (2/4; 50%), ST3 (1/4; 25%), and ST2 (1/4; 25%), together with an uncultured uncharacterized stramenopile. *G. duodenalis* genotype was AII (1/2; 50%).

**Conclusion**: We isolated intestinal protozoa from a super harsh condition, ORWWTPs. Our findings signify the concern due to the distribution of potentially pathogenic parasites to downstream lands via treated wastewater that may be released after treatment processing.

**Keywords**: Wastewater, intestinal parasites, Real-time PCR, subtyping, genotyping

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Investigation of the frequency of toxoplasmosis by ELISA method in HTLV1 positive people in Khorasan-Razavi province

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**Background**: Human T-cell lymphotropic virus type 1 (HTLV1) is a potentially serious and widespread viral infection in northeastern Iran that can disrupt immune regulation, and reactive latent infections such as *Toxoplasma gondii* cause severe immunologic complications. Therefore, we investigated concurrent infection with HTLV-1 and *T. gondii* in this area.

**Methods:** This study, in contribution with (ACECR)Academic Center for Education, Culture and Research branch of Mashhad, was performed on serum samples of 86 HTLV1- infected people. Serum samples of these patients were evaluated for the presence of anti-*T. gondii* IgG antibodies using the ELISA method. Serum samples from 91 non-HTLV1-infected individuals were also tested for the presence of anti-*T. gondii* IgG antibodies.

**Results:** In the HTLV1 positive group, 46 people (53.4%) were men and 40 people (46.6%) were women, and in the HTLV1 negative group, 53 people (58.2%) were men and 38 people (41.8%) were women. In terms of gender distribution, there was no significant difference between the HTLV1 positive and HTLV1 negative groups (p value=0.524). The anti-toxoplasma gondii IgG antibody was positive in the HTLV1 positive group in 66.7% of patients and in the HTLV1 negative group in 33.3% of cases, and there was a statistically significant difference between the two groups in this respect (p value>0.001).

**Conclusion:** The frequency of anti-Toxoplasma gondii IgG antibodies in the HTLV1 positive group is significantly different from the control group, and the amount of these antibodies was higher in the people infected with HTLV1 than in the group not infected with this virus. Therefore, it is very important to pay special attention to the reactivation of this parasite in people infected with HTLV1.

Keywords: Toxoplasmosis, HTLV1, Khorasan-Razavi province

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Poster

Investigation of the genomic prevalence of *Babesia* spp. in aborted sheep, Hamedan, Iran

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**Background**: Babesiosis, an important infectious haemoparasitic disease, is transmitted to livestock through ticks and causes significant direct and indirect losses to the livestock industry every year. *Babesia ovis* and *Babesia* mutasi are the most important species of *Babesia* found in the Middle East region.

**Methods:** 267 blood samples were taken from sheep that had aborted in the last 4 months. After DNA extraction of samples, the presence of *Piroplasma*, *B. mutasi* and *B. ovis* genes was detected by PCR, then by semi nested PCR method.

**Results**: In this study, 146 cases out of 267 (54.68%, CI95%: 48.71-60.65) and 140 cases out of 267 (52.43%, CI95%: 46.44-58.42) were infected with *Piroplasma* and *B. ovis*, respectively. No cases of *B. mutasi* were detected.

**Conclusion**: Based on the results, the prevalence of babesiosis in aborted sheep is high and despite the reports of the presence of *B. mutasi* in the western neighbors of Iran, *B. ovis* is considered the most common species in this region.

**Keywords**: *Babesia ovis*, *Babesia mutasi*, Hamedan,

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Investigation of the prevalence of intestinal parasitic diseases in patients with COVID-19 referred to teaching hospitals of Abadan University of Medical Sciences in 2022

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**Background**: Corona virus is a new pandemic disease that has caused widespread complications and deaths in the world, and it is necessary to identify the occurrence of infectious diseases at the same time as this disease and the factors affecting it.

**Methods:** This study was conducted on 234 patients with COVID-19. The information of registered people and the prevalence of intestinal parasitic diseases were also recorded. Then the results of this research were analyzed with SPSS software version 26 and with the help of *Chisquare* test. *P*-value less than 0.05 was considered significant in this study.

**Results**: The findings of our study showed that 76.1% of patients did not have parasites and 56 patients (23.9%) had parasites, including *Giardia* (8.1%), *Blastocystis hominis* (8.8%), respectively. 6%), *Entamoeba coli* (3.4%), *Cryptosporidium* (3%), *Entamoeba histolytica* (1.7%) and *Isospora* (0.9%). It was also found that the frequency of intestinal parasites in patients with COVID-19 with high school education, diabetes and hypertension, sedentary vegetable consumption and nausea and vomiting was significantly higher than other patients.

**Conclusion**: According to the obtained results, it can be seen that the prevalence of intestinal parasites in patients with covid-19 is high, and patients should be carefully examined and evaluated, and timely treatment will improve the quality of treatment and life of patients.

**Keywords**: COVID-19, Iran, outbreak, intestinal parasite

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Poster

Investigation of the prevalence of *Strongyloides* stercoralis and other intestinal parasites in patients with psychiatric disorders admitted to Sina Hospital in Hamedan in 1400

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**Background**: *Strongyloides stercoralis* is a parasite that is more common in people with immune system defects, psychiatric care centers, and prisoners. This study aimed to determine the frequency of intestinal parasites in patients with psychiatric disorders for health and treatment policy.

**Methods:** The study used a cross-sectional research method and focused on patients with psychiatric disorders who were admitted to Sina Hospital in Hamedan. The study aimed to detect *S. stercoralis* and other intestinal parasites in feces using a combination of direct observation, formal ether precipitation method, and modified agar plate culture.

**Results**: The study conducted on the stool samples of 370 patients suffering from psychiatric disorders and admitted to Sina Hospital in Hamedan revealed no infection with *S. stercoralis*. However, the study did find some patients infected with other parasitic agents, such as cysts of *Blastocystis hominis* (2.43%), *Giardia lamblia* (1.62%), *Entamoeba coli* (0.54%), and *Iodamoeba butschlii* (1%).

**Conclusion**: The lack of suitable weather conditions, the increase in the level of public health following the covid19 epidemic, and the possible anti-parasitic effects of antidepressant drugs can be effective in controlling and eliminating *S. stercoralis* in the target population.

**Keywords**: *Strongyloides stercoralis*, psychiatric disorders, strongyloidiasis

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Investigation of the students' attitudes towards online English lessons of parasitology in Isfahan University of **Medical Sciences** 

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Background: Parasitic diseases are one of the most important health challenges in most countries, especially developing countries. Virtual education is considered one of the most up-to-date learning environments in today's era. Knowing the level of preparation and attitude of these users is a necessity for the successful implementation of electronic learning.

**Methods:** This study is a descriptive-experimental research was conducted in Isfahan University of Medical Sciences with the participation of 198 students in the year 1400-1401. Demographic data and standard e-learning readiness questionnaire were used. Data analysis was done using descriptive statistics and inferential statistics (one-way ANOVA and t-test).

Results: Students have good preparation and attitude to participate in electronic learning of parasitology course. There is a meaningful difference between the student's level of knowledge and the title of the parasitology course. There is no significant difference between the level of readiness of students in different schools to participate in electronic learning. Also, comparing the motivation component with other components shows a decrease, which indicates that students have an average motivation to learn through learning.

Conclusion: The investigation of students' positive attitudes towards electronic English teaching of Parasitology indicates that this type of education can significantly contribute to their learning processes and increase the health level of the society. Also, a very favorable attitude towards e-learning environments is effective as an important educational tool for future.

Keywords: Attitude, parasitology, electronic learning, students

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Poster

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Investigation of tissue samples of patients with cancer for Toxoplasma gondii infection

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Background: Toxoplasma gondii is an intracellular parasitic protozoan that can cause severe complications and even death in immunocompromised patients, including cancer patients. The purpose of the present study is to investigate different cancerous tissues of patients in terms of T. gondii infection.

Methods: The study was conducted on 338 tissue samples of 9 types of cancerous tissue including lung, colon, breast, testicle, prostate, uterus, brain, ovary and eye. DNA was extracted from tissues and then PCR method was used to identify T. gondii using RE gene fragment as target with molecular weight of 529 bp.

**Results**: The frequency of infection with *T. gondii* among all types of cancer tissues was 33 cases (9.8%). The highest frequency of infection was related to lung tissue (38%) while the rest were colon (13.3%), breast (11.6%), testis (4.5%), prostate (3.3%) and uterus (2.6%). No infection was observed in brain, ovary and eye cancer tissues. From a statistical point of view, there was a significant relationship between the frequency of T. gondii infection and the type of tissues studied (P < 0.05).

Conclusion: The results of this study showed that Toxoplasma DNA is present in a significant number of cancer tissues of patients. In order to confirm the infection in the patients and to diagnose acute infection, it is necessary to perform serological tests to determine the titer of specific antibodies against Toxoplasma

Keywords: Toxoplasma gondii, cancer, prevalence, PCR

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Investigation of trichomoniasis in patients with vaginitis and its accompanying with other transmissible infections at northeast of Iran

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**Background**: Trichomoniasis is a common sexually transmitted disease (STD) caused by a parasite. In women, trichomoniasis can cause vaginitis, cervicitis and urethritis. In this study, the prevalence of *T. vaginalis* was investigated in individuals who referred to public health units in Mashhad.

**Methods:** Sampling was performed for women with signs and symptoms of inflammation. After DNA extraction, multiplex real time PCR was carried out for *Mycoplasma*, *Trichomonas*, *Niesseria*, *Clamidia*, *Gardanella*, *Ureaplasma*, HSV1&2 and the presence and relative level were measured by the FTD STD kit. For each person, a check list of background variables and history of disease was completed.

**Results**: The overall prevalence of *Trichomonas* infection was 4.17%. All of those infected were under graduated. The significant statistical correlation was not observed between trichomoniasis and other STD.

**Conclusion**: However, a relatively low prevalence of *T. vaginalis* infection was reported in this study, other laboratory tests are necessary to confirm the type of STD. Due to adverse outcomes of disease, public education is needed regarding implementation of personal hygienic measures and prevention of inappropriate sexual contacts.

**Keywords**: Trichomoniasis, vaginitis, STD infections, Iran

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Poster

Investigations effect of curcumin in PBMCs infected with *Leishmania major* [MRHO/IR/75/ER]

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**Background**: Leishmaniasis, which is caused by the *Leishmania* parasite, is considered a major tropical disease that is often neglected. The critical need for the discovery of efficient, cost-effective, and preferably herbal anti-*Leishmania*l agents is essential. Turmeric-derived curcumin is a natural polyphenolic compound renowned for its antioxidant, anti-inflammatory, and anti-tumor effects.

**Methods:** In this study, the anti-leishmanial activity of curcumin against *Leishmania major* was examined. Infected PBMCs were exposed to curcumin. The levels of ROS were monitored at 6, 12, and 24 hours, and gene expression levels were evaluated at 24, 48, and 72 hours following treatment with curcumin.

**Results**: According to the findings, the curcumin levels of 268  $\mu$ M [24 h] and 181.2  $\mu$ M [72 h] were identified as IC50 against *L. major* promastigotes. The treatment of *L. major* infected-peripheral blood mononuclear cells [PBMCs] with IC50 concentrations of curcumin led to a significant increase in the generation of reactive oxygen species [ROS] and upregulation of interferon-gamma [IFN- $\gamma$ ], tumor necrosis factor-alpha [TNF- $\alpha$ ], and nitric oxide synthase [iNOS] genes, depending on the duration of exposure.oxide synthase [iNOS] genes. These findings show the potential of curcumin against leishmaniasis.

**Conclusion**: The evidence presented in this research suggests that curcumin may hold promise in combating leishmaniasis.

Keywords: Curcumin, Leishmania major, PBMCs, gene

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Isolation and genotyping of *Acanthamoeba* species and Vahlkampfiidae in the harsh environmental conditions in the centre of Iran

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**Background**: Different species of free-living amoeba (FLA) have been abundantly isolated in harsh environmental conditions such as hot springs and brackish water. The present study aimed to isolate, genotype, and evaluate the pathogenicity of FLAs in Qom Roud, a large river, in the centre of Iran.

**Methods:** About 500 mL of water samples (n = 30) were collected from each sampling site and were investigated for the presence of FLAs using morphological and molecular characters. Genotype identification was performed using DNA sequencing and a phylogenetic tree was constructed with the MEGA X software. The pathogenic potential of all positive isolates was evaluated using the tolerance ability test.

**Results**: Morphological and molecular analysis indicated that 14 (46.66%) and two (6.66%) water samples were positive for *Acanthamoeba* species and Vahlkampfiidae, respectively. According to sequence analysis, *Acanthamoeba* isolates related to the T4 genotype and Vahlkampfiidae sequences were similar to *Naegleria philippinensis*. In the next step, thermo- and osmotolerance tests indicated four *Acanthamoeba* strains are extremely pathogenic. Our data showed the presence of potentially pathogenic *Acanthamoeba* T4 genotype and *N. philippinensis* in the super harsh Qom Roud. Contamination of water with virulent T4 genotype of *Acanthamoeba* may pose risk factors for contact lens users, children, and immunocompromised people.

**Conclusion**: The current findings serve as a document for the presence of Acanthamoeba species and Vahlkampfiidae amoeba in the salty water of Qom Roud. *Acanthamoeba* isolates belonging to the T4 genotype, which is implicated in the majority of *Acanthamoeba* infections. This river is related directly to human populations and further investigation

**Keywords:** Acanthamoeba, genotyping, Iran, Vahlkampfiidae

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Poster

Isolation and identification of *Acanthamoeba* strains from hemodialysis systems in Chaharmahal and Bakhtiari province using morphological and polymerase chain reaction methods in 2022

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**Background**: *Acanthamoeba* spp belong to Free-living amoebas that able to cause diseases such as meningoencephalitis, keratitis, especially in immunocompromised individuals. The presence of this potentially pathogenic amoebae in hospital environments is a source of infection. The aim of this study was to identify *Acanthamoeba* strains in hemodialysis systems.

**Methods:** In this descriptive cross-sectional study, 100 water samples were taken from 50 active hemodialysis machines (before and after washing and disinfection) in Chaharmahal-Bakhtiari province from July to February 2022, then them were investigated using culture on non-nutrient agar, microscopic observation, PCR and sequencing in order to evaluated contamination with *Acanthamoeba* spp. The obtained results were analyzed using SPSS version 26 software

**Results**: The frequencies of free-living amoebae were 48% in the samples of hemodialysis systems respectively, that *Acanthamoeba* spp were isolated and identified in 30% of hemodialysis samples. The results indicated that 20% and 10% samples of before and after washing and disinfection were infected with *Acanthamoeba* spp, respectively. In determining the sequence of the 7 *Acanthamoeba* samples, genotype T4 was identified. The statistical analysis of the results showed that there is no statistical relationship between the prevalence of contamination in hemodialysis system and the place (county), but there is a significant relationship between the sampling time and the prevalence of contamination.

**Conclusion**: The present study showed that *Acanthamoeba* is present in hemodialysis systems even after disinfection, and considering the pathogenic potential of *Acanthamoeba* in humans, therefore hemodialysis department personnels should receive the necessary training and awareness to reduce the frequency of *Acanthamoeba*.

**Keywords**: Acanthamoeba, hemodialysis systems, culture, PCR. **Correspondence Email(s):** r\_abdizadeh@yahoo.com


Isolation and molecular identification of Acanthamoeba spp. from hospital dust and soil of Khomein, Iran, as reservoir for nosocomial infection.

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**Background**: *Acanthamoeba* spp. are commonest opportunistic amoebae, which ubiquitous in various environmental resources. *Acanthamoeba* species are the causative agents of amoebic keratitis, granulomatous amoebic encephalitis and i.e. in immunocompromised and immunocompetent patients. Moreover, *Acanthamoeba* spp. can act as reservoir and transmission agent of bacterial pathogens.

**Methods:** In a cross sectional study, a total of 100 soil and dust samples were collected from hospital environment of Khomein Iran, and analyzed for the presence of *Acanthamoeba* spp. based on phenotypic and molecular methods including PCR amplification and sequence analysis of 18SrRNA. A total of 5 *Acanthamoeba* isolates were sequenced, and different genotypes of isolates were detected via direct sequence analysis.

**Results**: The results showed that 20% of samples (20/100) were positive for *Acanthamoeba*, while only 5 cases were successfully cultured in NNM medium and were subjected to molecular assay. *A. lenticulata*, *A. castellanii* and *A. quina* were the prevalent identified species that were belonged to T4 and T5 genotypes.

**Conclusion**: *Acanthamoeba* spp. are the most prevalent free-living amoeba in the dust and soil of hospital environment. Moreover, due to the presence of potentially pathogenic T4 genotypes in our hospital, it is recommended that in health and hygienic programs elimination of FLA should be considered.

**Keywords**: *Acanthamoeba*, phylogenetic, analysis hospital, environment

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Poster

Isolation and molecular identification of *Blastocystis* subtypes in human, livestock and birds in Kermanshah

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**Background**: The zoonosis hypothesis of the *Blastocystis* is always controversial and has not been definitively proven, therefore the present study was aim to identified *Blastocystis* subtypes among humans, livestock and birds of Kermanshah, Iran.

**Methods:** In the present study, fecal samples of cattle (220), sheep (240), and birds (140) were collected from the villages and cattle veterinary clinic of Kermanshah, Also 220 stool samples collected from patients were referred to the reference laboratory of Kermanshah city. The samples were examined by direct method, concentration (formalin-ethyl acetate) and culture. DNA extraction was performed using the Stool DNA Isolation mini kit (FavorGen, Taiwan) according to the manufacturer's recommended protocol. PCR amplification and sequencing of SSU rRNA gene were conducted with specific primers. Phylogeny analysis and haplotype network analysis of isolates performed with MEGA7 and PopART software.

**Results**: Twenty (9.1%) human isolates and two (1%) cattle isolates were positive according to microscopic survey. In total, 50 isolates including 10 human, 20 cattle, 10 sheep and 10 birds selected for molecular surveys. The results of sequencing and phylogeny analysis were identified ST2 and ST5 among human and animal respectively.

**Conclusion**: In the present study, ST2 and ST5 identified human and animals respectively. Since the prevalence of the parasite in human populations is significant, more accurate studies with a larger sample size and various genetic markers are needed to determine the epidemiology, zoonosis and pathogenicity of *Blastocystis*.

Keywords: Blastocystis, subtype, SSU rRNA, Kermanshah

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Knowledge, attitudes, and practices regarding cutaneous leishmaniasis among rural endemic communities in Esfarayen county, north Khorasan province, Iran

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**Background**: Cutaneous leishmaniasis (CL), a neglected tropical disease, poses a significant public health challenge in many endemic regions, including Esfarayen county in northeastern Iran. The knowledge and perceptions of individuals about leishmaniasis play a crucial role in disease prevention in these areas.

**Methods:** This community-based descriptive cross-sectional study utilized a quasi-experimental interventional design. A 65item questionnaire was developed to evaluate participants' knowledge, attitudes, and practices related to CL. Inferential statistics were conducted using independent samples t-tests, analysis of variance, and simple linear regression.

**Results**: Among the 250 participants, 47.2% were male and 52.8% were female, with a mean age of 33 ( $\pm$  13.85) years. Students accounted for 34.8% of the study population. The majority (90.4%) were aware of CL, and 91% could recognize its symptoms, typically presenting as cutaneous lesions. Over 60% of participants believed that mosquito bites, rather than sandflies, transmitted CL. However, attitudes towards CL treatment were found to be unsatisfactory. *Chi-square* tests revealed a significant association between participants' occupation and educational level with their awareness of the disease (P < 0.001).

**Conclusion**: The study underscores the importance of implementing effective health education programs to enhance residents' understanding of CL and their engagement in control initiatives within the region.

**Keywords**: Knowledge, attitudes, practices, cutaneous leishmaniasis

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Poster

 Latent toxoplasmosis frequency of men referred to Fertility and Infertility Research Center Hamadan University of Medical Sciences, Hamadan, Iran, 2023
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 Background: Toxoplasmosis is a parasitic disease caused by the intracellular protozoan Toxoplasma gondii. Some studies in

**Background**: Toxoplasmosis is a parasitic disease caused by the intracellular protozoan *Toxoplasma gondii*. Some studies in experimental models have reported the reduction of fertility factors and pathological changes in different male reproductive organs. This study aimed to determine the frequency of latent toxoplasmosis in men referred to Fertility and Infertility Research Hamadan University of Medical Sciences, Hamadan, iran.

**Methods:** This cross-sectional-descriptive study involved 197 male volunteers who referred to the Fertility and Infertility Research Center of Hamadan University of Medical Sciences for screening or investigation of infertility disorders. A commercial ELISA kit (Pishtaz Teb) was used to evaluate Sera were tested for anti-*Toxoplasma* IgG and IgM antibodies according to the manufacturer's instructions. Semen analysis as the cornerstone human male fertility was performed according to WHO guideline.

**Results**: Among 197 sample with an average age of 37.9 years (SD = 6.4) ranging from 23 to 60 years, 95 (48.22%) were positive for anti-*T. gondii* IgG antibody. Six (3%) of participants were positive for anti-*T. gondii* IgM. The volunteers who had fertility problems (181 people) were more than the people who were healthy fertile conditions (16 people) 91.9% versus 8.1%. The percentage (49.1%) of antibody against *T. gondii* was also higher in this group. Forty eight percent (48%) had problems in sperm morphology. These included defects in the head, neck and tail of the sperms.

**Conclusion**: This study suggests that although the effects of parasites on reproductive system is not fully understood, *T. gondii* can be considered as one of the male infertility factors.

Keywords: Toxoplasma, male, infertility

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Leishmanicidal, and cytotoxic effects Ferula macrecolea essential oil against Leishmania tropica

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**Background**: The current study was aimed to evaluate the chemical composition, as well as anti*Leishmania*l and cytotoxic effects of the essential oil of *Ferula macrecolea* and its main compound, terpinolene, against promastigotes and amastigotes of *Leishmania tropica*.

**Methods:** The chemical composition of the essential oil was analyzed by a gas chromatograph connected to a mass spectrometer (GC/MS). The MTT [3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromide] assay was used to study the effects of the essential oil and terpinolene against promastigotes while the macrophage model was used for evaluating the effect of *F. macrecolea* essential oil against amastigotes of *L. tropica* as well as assessing cytotoxicity. The Griess reaction assay was employed to study the nitric oxide (NO) produced by treating macrophage cells with the essential oil and terpinolene.

**Results**: The main compounds were terpinolene (77.72%), n-nonanal (4.47%), and linalool (4.35%), respectively. The 50% inhibitory concentrations (IC50) of the essential oil, terpinolene, and glucantime against promastigotes were 27.6, 11.6, and 32.8 µg/mL, respectively; however, their IC50 values against amastigotes were 42.3, 19.6, and 56.9 µg/mL, respectively. The 50% cytotoxic concentrations of the essential oil, terpinolene, and glucantime were 471.3, 207.3, and 1165.3 µg/mL, respectively. The production of NO in macrophage cells after treatment with the essential oil and terpinolene was increased in a dose-dependent manner (P < 0.001). The results revealed that by increasing the concentration of the essential oil.

**Conclusion**: *F. macrecolea* essential oil, especially its main compound, i.e., terpinolene, has a potent antiparasitic effect on the promastigote and amastigote stages of *L. tropica*.

**Keywords**: Leishmaniasis, promastigote, amastigote, nitric oxide

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Poster

Lipid based nanocarriers of cinnamon (*Cinnamomum zeylanicum*) oil for potential treatment of secondary hydatidosis

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**Background**: Cystic echinococcosis is considered as a neglected disease imposing a noticeable medical and economical challenges worldwide. Cinnamon oil (CO) has demonstrated potent antimicrobial effects even on hydatid cysts protoscoleces. This study aimed to synthesize cinnamon oil loaded Solid lipid nanoparticles (CO-SLNs) and to evaluate the protoscolicidal effects of this component.

**Methods:** CO-SLNs were prepared using emulsification, probe sonication technique, incorporating natural lipids (cholesterol and lecithin). SLNs were evaluated according particle size, poly dispersity index (PDI), zeta potential, electron microscopy, encapsulation efficiency (EE%), cell compatibility (MTT), etc. Scolicidal activity was assessed using eosin exclusion test (eosin 0.1%) with the concentrations of 0.5, 1,2,4,8 mg/mL of CO and CO-SLNs in time intervals of 10, 20, 60, 120, 180 minutes and 24 h.

**Results**: CO-SLNs analysis showed an average size of 337.6 with PDI 0.77 and zeta potential of -26 mV and EE of 83.49% with round morphology. MTT assay showed a higher cell viability in CO-SLNs compared to CO. The 100% mortality in PCs was observed in CO-SLNs with concentration of 8 mg/mL in 120 minutes and in CO with concentration of 8 mg/mL in 30 minutes.

**Conclusion**: CO-SLNs revealed a milder scolicidal activity which may be due to the sustain release of oil from SLNs which leads to a longer effective period of time, along with lower toxic effects on normal cells.

**Keywords**: Cystic echinococcosis, cinnamon, nanoparticles

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Medical parasitology is an endangered field in Iranian medical laboratories

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**Background**: Medical parasitology is the most important branches of medical science. Today, due to the progress of the health level and people's knowledge, there is a significant decrease in the incidence of gastrointestinal parasitic diseases.

**Methods:** This research is descriptive-analytical. The sample size was 50 medical diagnostic laboratories The data collection tool included a questionnaire, and the data collection included: the presence or absence of the diagnostic department of parasitology, the importance of this department, the use or non-use of standard diagnostic methods, and the presence or absence of experienced human resources. Finally, SPSS software was used to analyze the data.

**Results**: The results indicated that out of the 50 investigated laboratories; only 2 had a specialized section on medical parasitology. Out of these 50 laboratories, only 2 laboratories use one of the standard diagnostic methods for digestive parasites, and in 48 laboratories, the only diagnostic method is the direct examination of stool samples. Man power in 30 laboratories of laboratory science experts, and 20 laboratories of experts in the field of medical parasitology has been responsible for the medical parasitology department.

**Conclusion**: According to the results, it can be said that the reason for the decrease intestinal parasitic diseases, in addition to things such as the increase in the level of health and knowledge, can be caused by things such as the underestimation of the field of medical parasitology.

**Keywords**: Medical, parasitology, endangered, laboratories

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Poster

Modified method of tick processing for DNA extraction in order to evaluate the presence of pathogenic parasites

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**Background**: Due to the close association and relationship of ticks and parasitic disease, there is always a need to monitor and document ticks infesting human and animals and their associated tick-borne pathogens, especially using DNA based methods.

**Methods:** In our procedures, for DNA extraction, the oral appendages along with the salivary glands of the blood-fed ticks were separated. Then the hemolymph of the tick was removed from its outer shell (scotum) and separated so that the hard shell of the tick does not have an inappropriate effect on DNA extraction. The contents of hemolymph and oral appendages were placed in a 2 mL microtube. The contents of each microtube were frozen and thawed once in liquid nitrogen for 5 min, followed by 5 min in a water bath at 60 °C.

**Results**: Examining the obtained DNA using the Nadrop method and also electrophoresis on the gel showed that the DNA obtained by our modified method was of a higher quantity and a higher degree of purity.

**Conclusion**: The molecular isolation of pathogenic parasites that are transmitted by ticks requires the extraction of tick DNA with the appropriate purity and quantity, and since the outer shell of ticks does not play a role in the transmission of parasitic agents, it also interferes in the DNA extraction process.

Keywords: Tick, DNA, parasite

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Molecular and microscopic investigation of *Sarcocystis* species isolated from sheep muscles in Iran

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**Background**: *Sarcocystis* species is a genus of cyst-forming parasites infecting both humans and animals globally. Some of these species cause clinical and subclinical diseases in the host and may lead to economic losses. is study was carried out to identify the distribution patterns of *Sarcocystis* spp. in slaughtered sheep.

**Methods:** In total, 150 fresh muscle samples (30 hearts, 60 esophagi, and 60 diaphragms) were investigated by naked eye observation and then scrutinized based on the digestion method. To this end, pepsin and HCl were used to observe the *Sarcocystis* parasite via a light microscope. PCR was carried out to intensify a fragment of the 18S rRNA gene. Afterward, the PCR products were exposed to digestion by endonuclease TaqI, HindII, EcoRI, and AvaI. Consequently, the results of RFLP were confirmed by sequencing, and the phylogenetic placement of all species was analyzed.

**Results**: rough the examination by the naked eye, 5/150 (3.33%) macroscopic cysts were found in the samples. With the tissue digestion and microscopic examination, 116 (77.33%) samples were positive for *Sarcocystis* spp.; however, 125 (83.33%) samples were positive with PCR. Moreover, the results of sequence analysis on macrocysts and microcysts showed that 4% and 96% of the species belonged to *S. gigantea* and *S. tenella*, respectively. According to the results of the current study, sarcocystosis caused by S. tenella are highly prevalent among sheep in the Isfahan region.

**Conclusion**: Due to the high prevalence of *Sarcocystis* infection in the world and Iran, the development of disease control and prevention policies in sheep would be essential and changing attitudes in the way of keeping livestock from the traditional type to the industrial method is recommended in this regard.

Keywords: Sarcocystis, molecular, microscopic, sheep, muscles

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Poster

Molecular characterization of cathepsins gene of *Lucilia sericata* larvae (Diptera: Calliphoridae)

# Hamzeh Alipour<sup>1</sup> © **P**

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**Background**: *Lucilia sericata*, a Calliphoridae family member, is an important species used in larvae therapy. The larvae therapy method for the treatment of drug-resistant chronic ulcers, diabetic foot ulcers and bedsores was approved by the FDA in 2004. The larvae play a role in wound healing by secreting various enzymes.

**Methods:** The life cycle of *L. sericata* from egg to adult takes 10 days in laboratory conditions. RNA was isolated from larvae stage 2, and then cDNA synthesis was performed. Polymerase chain reaction (PCR) analysis of the synthesized cDNAs was performed with Gene-specific primers designed for the L. sericata cathepsin gene, and the obtained amplicons were cloned into the vector pTG19/blunt and the plasmid was extracted. Sequence confirmation of recombinant plasmids was determined with specific and universal (M13) primers.

**Results**: The 1002 bp nucleotide was deposited in the GenBank with the accession number PP108244. The CDS is 1020 bp long and is responsible for producing 399 amino acid residues with a predicted molecular weight of 37688.2 Da. The DNA sequence was compared in GenBank by BLAS and showed that it is 98.6% and 93.78% similar to *L.sericata* and *L.cuprina*, respectively.

**Conclusion**: It is assumed that this gene, which has woundhealing properties, can be used in future studies. The results of this study can be used by researchers worldwide to produce recombinant protein for wound healing.

Keywords: Cathepsins gene, Lucilia sericata, larvae

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Sth International & 12th National Congress of Parasitology and Parasitic Diseases of Iran Alborz University of Medical, Sciences, Karaj, Iran May 21 - 23, 2024

## Poster

Molecular detection of *Dientamoeba fragilis* in street and pet cats and their owners

Gita Alizadeh <sup>1</sup> @, Poorya Karimi <sup>1</sup>, Soheila Shafaghi-Sisi <sup>1</sup>, Ahmad Reza Meamar <sup>1</sup>, Elham Razmjou <sup>1</sup> ©

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**Background**: *Dientamoeba fragilis* is an anaerobic protozoan with global distribution in the colon of humans, non-human mammals, and birds. The limited investigations identified mammals and birds as natural hosts for D. fragilis. The identification and investigation of this protozoan in domestic animals has not been done in Iran.

**Methods:** This study was performed on the fecal samples of 132 street cats, 33 pet cats, and 33 cat owners in Tehran, Iran. The DNA of *D. fragilis* was identified by PCR targeting the 5.8S rRNA gene.

**Results**: The results of the investigations showed that 25 samples of street cats (18.93%), eight samples of pet cats (24.4%), and 11 samples of cat owners (33.3%) were infected with *D. fragilis*.

**Conclusion**: Our study is the first evidence of *D. fragilis* infection in small mammals in Iran, which introduces cats as a suitable reservoir for transmitting this parasite to humans. Our findings contribute to the human health approach by increasing awareness and knowledge about *D. fragilis* infections.

**Keywords**: *Dientamoeba fragilis*, cats, human, PCR

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Poster

Molecular detection of *Griardia duodenalis* in fecal samples from dogs in Hamedan, western Iran

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**Background**: *Giardia duodenalis* is a unicellular protozoan parasite being frequent in dogs. The infection is often asymptomatic but can cause diarrhea. Considering its zoonotic potential, the aim of this study was to determine epidemiological status of G. duodenalis in privately-owned and sheltered dogs in Hamedan city in western Iran.

**Methods:** During four seasons of 2022, a total of 348 fecal samples (87 samples in each season) were collected from privately owned (n = 158) and shelter (n = 190) dogs of both sexes, different ages and breeds in Hamedan city. Genomic DNA was extracted from the samples and tested for *G. duodenalis* DNA by targeting a 261 bp fragment of glutamate dehydrogenase (gdh) locus using conventional PCR. Statistical analysis was performed to identify risk factors associated with positivity.

**Results:** *Giardia* DNA was diagnosed in 136 dogs (39.1%). Keeping condition (P = 0.00001, higher infection rate in free-ranging dogs then being kept in NGO-run shelters) and season (P = 0.000034, highest infection rate in summer) were statistically recognized risk factors for fecal positivity.

**Conclusion**: This study provides the first information on the molecular prevalence of giardiosis in privately owned and free-ranging dogs from Hamedan. As dogs become infected with both zoonotic and non-zoonotic assemblages and genotypes of G. duodenalis, they may pose a risk to veterinary and public health in the region.

Keywords: Giardiasis, one-health, zoonosis

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Molecular detection of *Hepatozoon canis* in ticks collected from dogs in Kerman, Iran

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**Background**: Canine hepatozoonosis is widespread in some regions of Iran but there was no information on the prevalence and tick vectors of *Hepatozoon canis* in the southeastern city of Kerman. The aim of this study was to investigate the molecular prevalence of *H. canis* in ticks collected from dogs.

**Methods:** From 2019 to 2020, fifty tick-infested freeranging dogs in Kerman city (25 male, 25 female) were randomly chosen. From each dog, 5 engorged ticks were collected in individual labeled tubes and transferred to the laboratory. The ticks from each dog were morphologically identified, minced and pooled for genomic DNA extraction. Conventional PCR targeting an 897 bp fragment of the *H. canis* 16S rRNA gene was performed on 50 pools of tick, and products were gel-electrophoresed.

**Results**: All collected ticks were diagnosed as *Rh. sanguineus*. DNA of *H. canis* was detected in 14 tick pools (28%). The dogs from which positive ticks were collected were of both sexes and aged between 5 months and 6 years. This study demonstrates for the first time a substantial prevalence of *H. canis* in ticks feeding on stray dogs in Kerman posing a potential health risk to domestic and wild canids in the region.

**Conclusion**: We have shown that molecular testing of ticks for *Hepatozoon* DNA could be a suitable alternative to blood sampling in epidemiological studies. Effective strategies for ectoparasite control, regular examination of dogs and successful chemoprophylaxis are advocated.

Keywords: Canine, dog, brown dog tick

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Poster

Molecular diagnosis of toxoplasmosis using B1 & GR6 genes in pregnant and married women attending health centers of Shahid Beheshti University of Medical Sciences.

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**Background**: *Toxoplasma gondii* is a protozoan that can infect most warm-blooded vertebrates. It is transmitted through ingestion of oocysts or tissue cysts, causing toxoplasmosis with diverse manifestations. If a woman gets infected for the first time during pregnancy, the parasite can harm the fetus based on its genotype.

**Methods:** In this research, serum and blood samples were gathered from 240 women, including 132 pregnant and 108 married women. The ELISA kit from PISHTAZTEB DIAGNOSIS Co limited, known for its 100% sensitivity and 97% specificity, was employed to measure IgG and IgM antibody levels in these women. Seven samples tested positive for IgM, and their DNA was isolated using DNGTM-PLUS from Sina Clon BioScience Company. Subsequently, the B1 and GRA6 genes were amplified using a PCR machine, and all resulting products were sequenced.

**Results**: In the PCR method, we successfully amplified 5 positive IgM samples using the GRA6 gene and 7 positive IgM samples using the B1 gene, and all the isolated strains belonged to the type III genotype which is reported from different part of Iran.

**Conclusion**: In this study, all isolated samples were identified as type III, with no type II as the primary cause of human toxoplasmosis. Type III is the cause of severe diseases in immunocompromised adults, while type I is the main cause of disease in mice and immunocompetent adults.

Keywords: Toxoplasma gondii, genotyping, GRA6, B1

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Molecular diversity of cystic echinococcosis among surgical patients in Ardabil province, northwestern Iran

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Background: Cystic echinococcosis or hydatidosis is an important disease caused by Echinococcus granulosus metacestode. Human hydatidosis is considered an endemic disease in certain regions of Iran. The aim of this study was to determine the genetic diversity of E. granulosus in samples collected from different hospitals in Ardabil city.

Methods: A total of 228 human hydatid cysts (paraffinembedded or preserved in alcohol) that were surgically removed during 2014-2019 were obtained from hospitals of Ardabil. The cox1 and nad1 gene was amplified and sequenced from the extracted DNA. Phylogenetic and genetic diversity analysis was performed.

**Results**: The samples were from the cysts of the liver (73.4%), lung (20.3%), spleen (4.6%) and other organs (1.7%) of patients. Sequencing of cox1 and nad1 genes from 30 (13.1%) samples were done successfully. Genetic analysis showed that all isolates had G1 genotype.

Conclusion: The sheep strain (G1) play the main role in the transmission cycle of cystic echinococcosis in Ardabil province.

Keywords: Cystic echinococcosis, genetic diversity, Ardabil

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Poster

May 21 - 23, 2024

Molecular epidemiology of taeniidae infection with emphasis on Echinococcus granulosus in owner's dogs, using Copro PCR, in Semnan province Iran 2023

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Background: Echinococcus granulosus is one of the zoonotic helminthic infections that is important from the point of view of medicine and veterinary medicine in tropical and subtropical regions of Iran. This study aimed to determine the prevalence of E. granulosus infection among domestic dogs using the copro-PCR method in the Semnan.

Methods: 381 feces samples of domestic dogs were collected from different regions of Semnan province from May 2023 to February 2024. The coprological examinations were conducted by the formalin ether concentration method. The positive microscopic samples were accurately identified using two specific primers for the identification of taeniid and another set of primers was used for the specific diagnosis of E. granulosus sensu lato.

**Results**: A total of 381 faecal samples from owner's dogs were examined for the presence of parasites. Taeniid eggs were detected in 48 dogs (12.59%). All 48 taeniid-positive specimens were PCRpositive for cox1 (444 bp). Of all taeniid-positive specimens, 31 samples (8.13% of all dog specimens) were positive by E. granulosus specific primers.

Conclusion: The data presented in this research indicate a considerable prevalence of E. granulosus in owned dogs. The findings of this study are important from the point of view of epidemiology and hydatidosis control and prevention programs in endemic areas.

Keywords: Taenia, Echinococcus granulosus, Copro-PCR; COX1

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Molecular genotyping of *Giardia duodenalis* in humans in the Yazd county, central of Iran

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**Background**: Considering that there is no genetic data related to human giardiasis in central Iran, this study was conducted to identify assemblages and sub-assemblages of *G. duodenalis* collected from patients referred to Yazd Central Laboratory in central Iran.

**Methods:** Total of 35 fecal samples were collected from patients referred to Yazd central laboratory from February to July 2022. All the samples were included in this study after microscopic observation of *G. duodenalis*. DNA samples were extracted using related kit and were analyzed by Nano Drop. The molecular assessment was carried out using semi-nested PCR using the target gene of gdh. All amplified samples were sequenced using Sanger method. The sequences were analyzed by BLAST for assemblage identification.

**Results**: Out of 35 samples, 24 (68.57%) and 11 (31.43%) were male and female, respectively. All included samples were amplified using the specific gdh primer pair. The molecular analysis showed 17 isolates (48.57%) as assemblage BIV, 8 isolates (22.86%) as assemblage BIII, 6 isolates (17.14%) as assemblage AII and 4 isolates (11.43%) as assemblage AIII (p0.05).

**Conclusion**: The molecular analysis conducted in this study showed that assemblages A and B are the most prevalent in central Iran. The molecular identification of *G. duodenalis* isolates from animals and implementing control programs is strongly recommended as a preventive measure to limit the transmission of giardiasis to humans.

**Keywords**: *Giardia duodenalis*, genotyping, assemblages, Yazd

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Poster

Molecular identification and phylogenetic analysis of Leishmania major isolated from zoonotic cutaneous leishmaniasis foci in northeast Iran

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**Background**: Epidemiological studies, classification and genetic studies of *Leishmania* species are effective in treatment, control and prevention in endemic areas. We aimed to investigate the genetic diversity and phylogeny of *Leishmania* in zoonotic foci located in northeastern Iran using nagt gene for the first time.

**Methods:** DNA of 100 confirmed positive slides collected from the health centers of Sarkhes, Darghez, Fariman, Esfarayen, and Sabzevar were extracted during 2020-2021. The partial sequence of kDNA was amplified to identify the species. Twenty-five DNA samples were randomly subjected to amplify by nagt gene primes and were sequenced. The sequences were aligned with reference sequences in National Center for Biotechnology Information (NCBI). Then, the genetic similarities of the sequences were checked using Clustalx 2.1 software and the phylogenetic tree was drawn by Mega 7 software.

**Results**: All the positive samples were diagnosed as *L. major*. Approximately, half of the sequences of species were similar to two reference genes JX103550.1:404-712 *L. major* Esfahan and KX759012.1:568-807 *L. major* Ilam (more than 90% similarity). According to the results of the phylogeny tree, the closest genotype to our study samples was JX103550.1:404-712 *L. major* Esfahan.

**Conclusion**: The most causative agent CL in these areas was *L. major*. The genetic diversity of *L. major* was high such as other zoonotic foci in Iran. Due to the high similarity of the strains in the study areas with the strains of Isfahan and Ilam, similar control and prevention methods.

**Keywords**: Iran, *Leishmania*, northeastern, phylogeny, zoonotic

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Molecular identification of *Anaplasma* species in slaughtered cattle in Hamedan, west of Iran

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**Background**: *Anaplasma* species are intracellular gramnegative tick-borne bacteria parasitizing host blood cells. Several species including *A. marginale*, *A. bovis*, *A. centrale*, *A. phagocytophilum*-complex and *A. platys*-complex infect bovines. This study aimed to investigate the prevalence of *Anaplasma* species in cattle of Hamedan city in west of Iran.

**Methods:** During the winter of 2022, blood samples were collected from 70 apparently healthy Holstein (n = 19), native (n = 11) and cross-breeds cattle (n = 40) of both males (n = 43) and females (n = 27). Genomic DNA was extracted and examined for *Anaplasma/Ehrlichia* species. Positive samples were further tested with species-specific primers targeting above mentioned *Anaplasma* species by conventional PCR method.

**Results:** Screening PCR revealed infection of 17 cattle (24.3%) with *Anaplasma/Ehrlichia* species. Subsequent species-specific PCRs showed DNA of *A. centrale* in 3 (4.29%), *A. marginale* in 1 (1.43%), *A. bovis* in 1 (1.43%), and *A. platys*-complex in 1 (1.43%) cattle. Mixed infection with A. centrale + A. marginale and A. centrale + A. platys-complex was detected in two samples. DNA of *A. phagocytophilum* and related strains (*A. phagocytophilum*-like 1 and *A. phagocytophilum*-like 2) was not detected.

**Conclusion**: This preliminary study indicated circulation of 4 *Anaplasma* species in cattle population in the region. Considering economic impact of subclinical infections and vertical transmission of some *Anaplasma* species regular examination of cattle and successful chemoprophylaxis are advocated.

Keywords: Anaplasmosis, PCR, ruminant Correspondence Email(s): aliasghar.bahari@basu.ac.ir Poster

Molecular identification of *Echinococcus* genotypes using pathological specimens of patients operated in Ayatollah Mousavi Hospital of Zanjan during 1391-1400

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**Background**: Cystic echinococcosis is one of the most prevalent parasitic diseases in both humans and animals. This disease is caused by the larval stage of *Echinococcus granulosus*. This study aimed to genotyping of hydatid cysts using pathological samples from patients operated on in Ayatollah Mousavi Hospital, Zanjan during 1391-1400.

**Methods:** In this cross-sectional study, 50 formalin-fixed, paraffin-embedded tissue samples from patients who underwent surgery at Ayatollah Mousavi Hospital, Zanjan were collected and analyzed by PCR-RFLP of ITS1 gene using Rsa1 enzyme and sequencing of a fragment of cox1 gene. The sequenced fragments were used for phylogenetic analysis.

**Results**: Using PCR-RFLP of ITS1 fragment, all 50 isolates were belonged to *E. granulosus* senso stricto (G1-G3 genotypes). Sequence analysis of mitochondrial cox1 fragment of 9 cyct isolates revealed that all of them belong to the G1 genotype of *E. granulosus*. These 9 isolates were divided under 3 different sequence types and their cox1 sequence were most similar to hydatid cysts isolated from different intermediate hosts in Turkey and Portugal.

**Conclusion**: The predominant genotype of human cystic echinococcosis in Zanjan region is G1 (common sheep strain), as in most parts of Iran. Among the cox1 sequence types (STs) obtained in this study, two STs have been previously reported and another ST is reported for the first time.

**Keywords**: *Echinococcus granulosus*, Cox1, Zanjan, genotype

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5th International & 12th National Congress of Parasitology and Parasitic Diseases of Iran			
	Alborz University of Medical, Sciences, Karaj, Iran		
	May 21 - 23, 2024		
Poster	Poster		
Molecular identification of pathogenic free-living amoeba from household biofilm samples in Iran: a risk factor for <i>Acanthamoeba</i> keratitis	Molecular identification, phylogenetic analysis and networking of human <i>Trichostrongylus</i> species fr endemic area of Iran		
Maryam Norouzi <sup>2</sup> @, Maryam Niyyati <sup>4</sup> ©, Reza Saberi <sup>2</sup> , Hamed Mirjalali <sup>3</sup> , Marziye Fatemi <sup>1</sup> , Ehsan Javanmard <sup>4</sup> , Seyed Ahmad Karamati <sup>5</sup>	Hamed Mijalan <sup>+</sup> ©, Sara Nemati <sup>+</sup> @, Meysam sha Hanieh Mohammad Rahimi <sup>1</sup>		
<ol> <li><sup>1</sup> Department of Medical Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran</li> <li><sup>2</sup> Department of Medical Parasitology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran;</li> <li><sup>3</sup> Foodborne and Waterborne Diseases Research Center</li> </ol>	<sup>1</sup> Foodborne and Waterborne Diseases Research Research Institute for Gastroenterology and Liver Shahid Beheshti University of Medical Sciences, Tel <sup>2</sup> Department of Medical Parasitology and Mycolog of Medicine, Guilan University of Medical Science Iran		
<ul> <li><sup>4</sup> Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran</li> <li><sup>5</sup> Department of Medical Parasitology and Mycology, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad</li> </ul>	<b>Background</b> : Human infections with <i>Trichostrongylus</i> a zoonotic disease that has been reported in most parts of aim of this study was the identification, molecular charac networking analysis and phylogenetic analysis <i>Trichostrongylus</i> species based on cytochrome c oxidas (COX1) from Guilan province, northern Iran.		
University, Tehran, Iran Background: Free-living Amoeba (FLA) are distributed in the environment. A biofilm comprises any microorganisms in which cells stick to each other and also to a surface. This study aimed to check for the presence of FLA in samples from household biofilms	<b>Methods:</b> A total of 206 fresh stool samples were coll residents of endemic villages of Northern Iran. All sar examined using conventional parasitological methods, the PCR technique. In order to illustrate the relationship, haplotype network and molecular diversite travelogicate and using applied part actuary between the set of		

Methods: A total of 69 biofilm samples collected from showerheads, kitchen areas, and bathroom sinks were analyzed. Positive samples for FLA were characterized at the morphological and molecular levels.

in Iran and to characterize them at the molecular level.

Results: The results of morphology analysis indicated that 26.08% (18/69) of biofilm samples were positive for Acanthamoeba spp., Vermamoeba genus, and Vahlkampfiids. According to sequence analysis, five strains of Acanthamoeba isolates related to the T4 genotype and two strains belonged to the T2 genotype. In addition, the pathogenic potential of Acanthamoeba-positive isolates was conducted using the tolerance ability test. The results of BLAST of Vermamoeba sequences were similar to what was expected for Vermamoeba vermiformis.

Conclusion: The reasons revealed that relative contamination of household biofilm samples with FLA may pose a risk for people using soft contact lenses and with traumatic cataract. Our finding proposes that filtration should be performed in shower heads and indicates the need to monitor people at increased risk of Acanthamoeba keratitis.

Keywords: Free living amoeba, biofilm, genotyping, Iran

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haplotype om an

rifdini<sup>2</sup>,

Center. Diseases, hran, Iran y, School s, Rasht,

s species is of Iran. The cterization, of human e subunit I

ected from nples were along with phylogeny ty between Trychostrongylus spp., were applied PapArt networking, DnaSP v.6 and MEGA 10 software.

**Results**: In total, from 206 fecal samples, 71 people (34.4%) were found infected with Trichostrongylus spp. The ~700-bp fragment of the COX1 was amplified in all 71 morphological positive samples, however, 33 samples were successfully sequenced and belonged to the genus Trychostrongylus spp. In this study, T. colubriformis was the predominant species and one sequences were characterized as T. vitrinus. Interestingly, our T. vitrinus sequence was grouped together with T. vitrinus sequences, which were obtained from animal in Guilan province (97.17% similarity). In total, 26 haplotypes were identified and Haplotype diversity (Hd) ranged from  $0.988 \pm 0.012$ .

**Conclusion**: Due to the importance of trichostrongylosis in public health as a zoonotic infection, having information about the prevalence of *Trichostrongylus* in animals and humans in a region can provide valuable information about the transmission cycle of different species of this parasite between humans and animals.

Keywords: Trichostrongylosis spp., cytochrome c oxidase subunit

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Molecular investigation of *Cytauxzoon* infection in owned and stray cats in Tehran

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**Background**: Cytauxzoonosis, an emerging tick-borne disease of domestic cats and wild felids, is caused by the protozoa belonging to the genus *Cytauxzoon*. *Cytauxzoon felis* cause rapidly progressive fatal disease in domestic cats. Infected domestic cats show clinical signs such as pyrexia, anorexia, dehydration, depression, icterus, and hepatosplenomegaly.

**Methods:** This study was performed to determine the frequency of *Cytauxzoon* infection in owned and stray cats in Tehran city. Blood samples were collected from 90 stray and owned cats referred to the Central Veterinary Hospital of Tehran. Microscopic evaluation was performed by preparation of Giemsa stained blood smears. DNA extraction of blood samples, including suspected samples by microscopic examination, was done using MBST kit. All samples were tested for *C. felis* infection using a polymerase chain reaction assay base on 18S rRNA gene.

**Results**: No blood sample was PCR-positive for *C. felis*. According to our results, it seems that microscopic method has low specificity in diagnosis of *C. felis*.

**Conclusion**: Due to the limited number of cats in this study, the results of this study cannot be attributed to the cats of Tehran city. However, it is likely that *C. felis* is absent among carts in Tehran or the infection rate is low.

Keywords: Cytauxzoon, 18S rRNA, cat, Tehran

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Poster

Molecular prevalence of Cryptosporidium spp. in cattle

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**Background**: *Cryptosporidium* is a common zoonotic enteric protozoa that causes clinical and subclinical infections in animals and humans worldwide. The high prevalence of this parasite in cattle suggests a significant transmission risk to humans. Therefore, the study aimed to investigate the prevalence of *Cryptosporidium* spp. in cattle.

**Methods:** The study was performed on 200 fecal samples of cattle. Nested-PCR of the small subunit ribosomal RNA (rRNA) gene was conducted to screen for *Cryptosporidium* spp., followed by sequencing in both directions to identify *Cryptosporidium* species. Nucleotide sequences of isolates aligned with the sequences in the GenBank database and the phylogenetic analysis was performed.

**Results**: *Cryptosporidium* was identified in 79.5% (159/200) by 18s rRNA gene amplification. The sequencing analysis identified *C. andersoni*, *C. bovis*, *C. ryanae*, *C. parvum*, and *C. suis*/ occultus in 30, 25, 15, 13, and 2 cattle. There was a significant association between the prevalence of *Cryptosporidium* spp. and age, with the highest prevalence (52.9%) in the 1 to 6-month age cattle.

**Conclusion**: The high prevalence of *Cryptosporidium* infection (79.5%) in cattle suggests their essential role as a potential reservoir for this parasite. Therefore, accurately identifying and treating *Cryptosporidium* infection in cattle is necessary to reduce environmental contamination by releasing oocysts that may infect humans through soil or water contamination.

Keywords: Cryptosporidium, cattle, Iran, 18s rRNA

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Molecular survey and genotyping of *Toxoplasma gondii* in paraffin-embedded blocks of patients with brain tumor refferd to Mazandaran Comprehensive Cancer Center

Seyed Abdollah Hosseini <sup>1</sup> © Ø, Shahabeddin Sarvi <sup>1</sup>, Mitra Sadeghi <sup>1</sup>, Seyed Ali Shariatzadeh <sup>2</sup>, Bahareh Basirpour <sup>3</sup>, Misagh Shafizad <sup>4</sup>, Amir Reza Beh Rouzi <sup>5</sup>, Elham Miraboutalebi <sup>5</sup>, Fattaneh Montazeri <sup>3</sup>, Ehsan Zaboli <sup>6</sup>, Farshad Naghshyar <sup>7</sup>

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**Background**: *Toxoplasma gondii* is high prevalent in Mazandaran province. Different types of *T. gondii* can cause asymptomatic or fatal forms of toxoplasmosis in cancer patients. This study aimed to investigate the molecular and genotyping of *T. gondii* in brain tumor patients referred to the Cancer Center of Imam Khomeini Hospital (CCIKH).

**Methods:** A total of 50 blocked tissue samples of people with brain tumor admitted to the CCI KH in Sari in Mazandaran province were included in the study. Next, DNA extraction was done and PCR test was performed using RE gene. Finally, *Toxoplasma* genotyping was performed using nested-PCR and after sequencing, a phylogeny tree was drawn.

**Results**: Out of the total people with cancer, 29 were men and 21 were women. The results of the molecular test showed that the DNA of *Toxoplasma* was higher in men (8 positive cases out of 29 samples, 27.59%) than in women (3 positive cases out of 21 samples, 14.29%). Molecular results showed that 22% (11) of people with brain tumor had *Toxoplasma* DNA. In total, 63.63% of the samples had genotype I and the remaining samples had genotype II.

**Conclusion**: This study showed that patients with brain tumors in northern Iran are at risk of acute toxoplasmosis, including toxoplasmic encephalitis. Due to the high prevalence of *T. gondii* in this province and the significant prevalence of pathogenic type I, prevention measures are necessary to avoid irreversible complications in these patients.

Keywords: *Toxoplasma gondii*, genotyping, brain tumor Correspondence Email(s): hosseini4030@gmail.com

#### Poster

Molecular survey of *Toxoplasma gondii* in lymph nodes of patients with Lymphadenopathy in Mazandaran province

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**Background**: Toxoplasmosis is one of the causes of lymphadenopathy but toxoplasmic cysts are rarely found in the histological diagnostic sections. However, the validity of the histopathological triad is confirmed by serological tests. Now, the presence of *T. gondii* DNA in the tissue can be detected by PCR method.

**Methods:** This study was conducted on the 100 tissue samples of the lymph nodes of people with lymphadenopathy referred to the hospitals of Mazandaran province. Tissue was extracted from the paraffin blocks and then Parasite DNA was extracted using a Tissue DNA extraction kit. Out of 100 case of lymphadenitis, 34 cases were related to acute lymphadenitis and 66 cases were chronic lymphadenitis. The lymph nodes were biopsied from the axilla, neck, inguinal and tonsillar. Nested-PCR method was done to amplify the coding region of the GRA6 gene. PCR products corresponding to 164 bp of the normal control DNA were posetive.

**Results**: Four samples out of 100 lymph nodes (4%) were positive for *T. gondii* infection, 2 cases belonged to acute lymphadenitis (2/34, 5.89 %) and 2 cases belonged to chronic lymphadenitis (2/64, 3.03 %). The results of the present study show that most of the *Toxoplasma* samples isolated from people with lymphadenopathy were of genotype II. Genotypes I and II were identified in 1 and 2 positive *Toxoplasma* samples in Mazandaran province, respectively.

**Conclusion**: This study showed that along with pathological tests in cancer patients, especially in breast cancer, molecular tests should be used to diagnose toxoplasmic lymphadenitis. It is suggested that researchers perform pathological, serological and molecular studies simultaneously in people suspected of lymphadenopathy to reduce the possibility of false positives and negatives.

**Keywords**: *Toxoplasma gondii*, toxoplasmosis, lymphadenopathy, genotyping

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Morphometric and molecular identification of *Fasciola* spp. in livestock from northwestern provinces of Iran

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**Background**: Fascioliasis is a zoonotic parasitic disease with worldwide distribution caused by the liver flukes, *Fasciola hepatica* and *Fasciola gigantica*. Accurate identification of *Fasciola* species is necessary to understand the epidemiological pattern of fascioliasis. This study aimed to determine the morphometric and molecular characterization of *Fasciola* spp. in livestock from northwestern.

**Methods:** Five hundred Adult Fasciola flukes were obtained from infected livers (n = 100) of different definitive hosts (cattle, sheep, goats, and buffaloes) in four local abattoirs in the northwestern provinces of Iran (West-Azerbaijan, East-Azerbaijan, Ardabil, and Zanjan) from September 2021 to August 2022. Then, all samples were identified based on the morphometric criteria and molecular methods using PCR-RFLP of ITS1 region with RsaI restriction enzyme. The results were analyzed using independent T-test and ANOVA using SPSS software. PCR-RFLP methods compared morphometric results of *Fasciola* samples. The Cox1 gene sequences were used to confirm PCR-RFLP results and phylogenetic analysis.

**Results**: Based on morphometric criteria, out of 500 *Fasciola* species, 139 samples (27.8%) were identified as F. gigantica and 361 samples (72.2%) were identified as *F. hepatica*. Similarly, PCR-RFLP analysis of the ITS1 region confirmed the morphometric results. The intermediate form of *Fasciola* was not identified. In this study, using Cox1 partial sequences, it showed 13 variable regions with eight haplotypes in *F. hepatica* and 12 variable regions with five haplotypes in *F. gigantica*. The identity and divergence percentages for *F. hepatica* were 98.2-100% and 0-2.3, respectively. Also, the percentage identity and divergence for *F. hepatica* were 97.8-100% and 0-2.2, respectively.

**Conclusion:** PCR-RFLP method can be used to confirm the morphological identification method of *Fasciola* species, but it is insufficient to study their genetic diversity. Also, the partial cox1 gene sequence results showed that *F. hepatica* and *F. gigantica* in the northwestern provinces of Iran have different genetic structures and haplotypes.

Keywords: Fasciola, RFLP, Cox1, phylogenetic, Iran

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## Poster

Naloxone effects against cutaneous leishmaniasis caused by *Leishmania major* strain MRHO/IR/75/ER in the BALB/c Mice

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**Background**: Cutaneous leishmaniasis (CL) is a serious health problem. Since the use of pentavalent antimonial compounds for the treatment of CL has side effects, naloxone as a new treatment in the footpad of *Leishmania major* (*L. major*)-infected BALB/c mice was investigated by evaluating the lesion size and the parasite burden.

**Methods:** The animals were infected with *L. major* (MRHO/IR/75/ER). 40 BALB/c mice were divided into 4 groups (10/group), and were treated as follows 39 days after *L. major* infection: Group 1 treated with intraperitoneal injections of MA (100 mg/kg, positive control group) daily for six weeks; Group 2 received a 100  $\mu$ L injection of PBS (negative control group); Group 3 received subcutaneous (SC) injections of naloxone (10 mg/kg) daily for six weeks (Naloxone1), and Group 4 was SC injected with naloxone (10 mg/kg) weekly for six weeks (Naloxone2). The lesion size was measured using a digital caliper.

**Results**: After the end of treatment, the lesion parasite burden was evaluated. As compared to the negative control group, the groups that received MA and naloxone (groups 1, 3, and 4) showed fewer parasites. Also, the naloxone-treated mice showed significantly smaller lesion sizes than the negative control group (P < 0.05), but they did not differ significantly from the MA-treated mice.

**Conclusion**: Taken together, the results suggest that naloxone might be a promising and alternative treatment for CL.

Keywords: Naloxone, *Leishmania major*, cutaneous leishmaniasis

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## Poster

Nanoemulsion of *Cinnamomum zeylanicum* essential oil shows potent anti-leishmanial activity

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**Background**: Leishmaniasis is a vector-borne disease caused by the *Leishmania* genus, a mandatory intracellular parasite. Cutaneous Leishmaniasis is the most common form of *Leishmania*sis; 2 million cases are infected with this disease annually, and 350 million people in 102 countries are at risk of infection.

**Methods:** The ingredients of *Cinnamomum zeylanicum* essential oil were investigated using GC/MS analysis. After that, its nanoemulsion dosage form was prepared using spontaneous emulsification. Besides, the leishmanicidal effect of the essential oil against promastigotes of *L. major* and *L. tropica* was investigated.

**Results**: Cinnamaldehyde, with 62.04%, was its major component. Linalool (6.96%), trans-caryophyllene (6.60%), trans-cinnamyl acetate (4.29%), and benzyl benzoate (3.32%) were other major constituents. Half-maximal inhibitory concentrations (IC50)s of the essential oil against *L. major* and *L. tropica* were 16.53 and 7.56 µg/mL. The nanoemulsion was prepared with a 52  $\pm$  4 nm droplet size and a low droplet size distribution index (SPAN = 0.97). Interestingly, after treatment of both species with nanoemulsion, their viability was reduced to 0%.

**Conclusion**: The prepared nanoformulation could be considered a potent anti-leishmanial agent for other species and in vivo studies.

**Keywords**: Nanoemulsion, cutaneous leishmaniasis, *Cinnamomum zeylanicum* 

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Poster

Nanoliposomes increases Anti-*Trichomonas vaginalis* and apoptotic activities of metronidazole

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**Background**: Trichomoniasis, caused by *Trichomonas vaginalis*, is the most common non-viral sexually transmitted disease worldwide. As current trichomoniasis chemotherapies have many side effects, we examined the Anti-Trichomonas effects of nano-liposomal metronidazole (NLMTZ) compared to metronidazole (MTZ) in vitro. **Methods:** Liposomes were produced using the thin film hydrationsonication technique with a slight modification coated with MTZ. The average hydrodynamic diameter of monodispersed NLMTZ was evaluated by DLS and the morphological measurements were performed by scanning electron microscopy (SEM). The effects of NLMTZ and MTZ (5, 10, 20 and 40 µg/mL) on *T. vaginalis* trophozoites (105 cells/mL) in trypticase-yeast extract-maltose (TYM) medium were evaluated in different exposure times. Then, cell viability, IC50, SEM analysis and the expression of the metacaspase gene were assessed by qRT-PCR.

**Results**: Growth inhibition of MTZ in a concentration of 40 µg/mL was 39.34% after 3 h, whereas NLMTZ caused 51% growth inhibition after 3 h and lysed Trichomonas completely after 12 h. The IC50 values were estimated at 31.51 and 15.90 µg/mL after a 6 h exposure for MTZ and NLMTZ, respectively. Moreover, both *T. vaginalis* treated with MTZ and NLMTZ had high levels of metacaspase mRNA expression relative to the control groups (P < 0.05). A significant difference was observed between the apoptotic intensities of *T. vaginalis* treated with MTZ and NLMTZ (P < 0.05).

**Conclusion:** This study showed that nano-liposomal MTZ is a potentially excellent approach for the treatment of trichomoniasis in vitro, although further studies are needed before consideration of clinical trials.

**Keywords**: Apoptosis, liposome, metacaspases, metronidazole, *Trichomonas* 

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New morphological approach to rapid identification of *Trichuris skrjabini* and *Trichuris ovis* 

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**Background**: Trichuris is one of the most common gastrointestinal nematodes found in the large intestine of small ruminants around the world. The most prevalent species in Iran are *Trichuris skrjabini* and *Trichuris ovis*. The objective of present study is morphological analysis of ovine Trichuris species in different geographical provinces of Iran.

**Methods:** From July 2022 to Feb 2023, 1100 cecum and colon of sheep/goat slaughtered at slaughterhouses in various regions of Iran including: Hamedan, Tehran, East and West Azerbaijan, Kerman, Sistan and Balouchestan, Mazandaran, Hormozgan, Fars and Zanjan provinces were inspected. The collected male and female *Trichuris* nematodes were examined based on morphology and morphometry. The general appearance of specimens was scanned by the PathScan enable IV instrument. The dimensions of the worms were measured using a calibration slide and Image J software (V.1.52). The SPSS software (V.27) and One-way ANOVA method was used to compare the results.

**Results**: The results of the present study revealed two significant differences in A: anterior/whole body length ratio in females of *T. skrjabini* (0.702  $\pm$  0.058) compare to *T. ovis* (0.624  $\pm$  0.191) and B: The average posterior width length in the males of *T. skrjabini* (1076.63  $\pm$  218.52 µm) compared to *T. ovis* (1330.19  $\pm$  344.44 µm).

**Conclusion**: However, there are some morphological parameters based on reproduction system in Trichuris species identification, the present study illustrate new morphological approach to rapid identification of *T. skrjabini* and *T. ovis* in Iranian small ruminants which can be helpful in early and quick identification of these two species.

Keywords: Ruminants, Trichuris, morphology, morphometry

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### Poster

Nosocomial diarrhea distribution by *Cryptosporidium* spp. in central Iran

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**Background**: *Cryptosporidium* spp. is a parasite that causes a human and animal diarrheal disease known as cryptosporidiosis. It is commonly transmitted through contaminated water, food, and surfaces contaminated with feces from infected humans or animals. Cryptosporidiosis is a serious public health and environmental challenge, so most studies focus on vulnerable groups.

**Methods:** This study examined 217 patients admitted to the hospital who experienced symptoms such as heartburn and acute or chronic diarrhea within 48-72 hours of admission. The modified Ziehl Neelsen (mZN) stain was used microscopically to recognize *Cryptosporidium* spp., and the 18S rRNA gene was amplified by nested-PCR.

**Results**: *Cryptosporidium* spp. prevalence was estimated to be 0.00% (0/217) by microscopy; however, PCR results indicate that the prevalence is 0.92% (2/217) in fecal samples.

**Conclusion**: The distribution of nosocomial diarrhea specifically caused by *Cryptosporidium* spp. is uncommon. It is imperative to note that healthcare settings, including hospitals, can be potential sources of transmission of various infectious diseases, including cryptosporidiosis. Proper infection control measures, including hand hygiene, disinfection, and water treatment, are crucial in preventing *Cryptosporidium*.

**Keywords**: *Cryptosporidium*, nosocomial diarrhea, opportunistic infection

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Occurrence of *Cryptosporidium* oocysts in vegetables consumed within cosmopolis Yazd, Iran

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**Background**: *Cryptosporidium* is a coccidian parasitic Protozoa that causes the diarrheal disease cryptosporidiosis. Outbreaks of human parasitic diseases associated with the consumption of raw vegetables. In the present study carried out Occurrence of *Cryptosporidium* oocysts in vegetables consumed within Cosmo polis Yazd, Iran.

**Methods:** A survey was carried out using 270 fresh raw vegetable samples in Yazd, Iran, for revealing of *Cryptosporidium* oocysts in vegetables using sucrose flotation medium of 1.21 specific gravity and modified Ziehl Nielsen staining procedure.

**Results**: 85 (31.5%) out of the 275 vegetables examined were positive for *Cryptosporidium* and 190 (68.5%) vegetables were negative for *Cryptosporidium*. Lettuce had the highest 16(47.1%) contamination rate followed by Radish14 (46.7%), Spring onion 11(35.5%), Leek 9 (30%0), Mint and Coriander 8 (26.7%), Tarragon 7(23.3%) Basil and Parsley are the lowest 6 (20%) contamination. There was statistical significance ( $X^2 = 15.487a$ , DF = 8; P = 0.049) between occurrence of *Cryptosporidium* and types of vegetables examined. Occurrence of *Cryptosporidium* oocytes in the different vegetables fields showed that Amir Abad had the highest 16 (59.3%) contamination rate followed by Ghiam Square 14 (51.9%), Imam Shahr 12 (44.4%).

**Conclusion**: Based on our results, edible vegetables in Yazd city are one of the most the potential sources *Cryptosporidium* infection in human and vegetables fields within Yazd city (Iran), are contaminated with *Cryptosporidium* that is community health consequence.

Keywords: Raw vegetables, *Cryptosporidium* oocytes, Yazd,

**Correspondence Email(s):** fatemehkuchakzade@gmail.com Poster

Occurrence of *Echinococcus granulosus* and *Toxocara* spp. in vegetables, Yazd, Iran in 2023

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**Background**: Vegetables, essential for a balanced diet, offer vital nutrients. However, unwashed raw vegetables can harbor pathogens and zoonotic parasites like *Toxocara* and *Echinococcus*. In this study, we examined parasite contamination in vegetables collected from Yazd city, Yazd province, Iran in 2023.

**Methods:** In 2023, a descriptive study was conducted in Yazd city, involving the random collection of 250 lettuce and vegetable samples from five regions. Following traditional cleansing, samples were divided into 100g portions, treated twice with a 5% Tween 80 solution. Resulting sediments were transferred to Falcon tubes and subjected to centrifugation at 2000 rpm for 10 min. The flotation method, employing a saturated ZnCl2 solution, concentrated and recovered parasite eggs. Microscopic examination, using 10x and 40x lenses, was conducted in a parasitology laboratory. *Echinococcus* spp. was identified using PCR and sequencing with sequence analysis performed using BLAST for confirmation.

**Results**: Out of 250 vegetable and lettuce samples 44 samples (17.6%) were contaminated with *Toxocara* spp. and Echinococcus *granulosus*. Among them, 34 samples (13.6%) were contaminated with *Toxocara* spp. and 10 samples (4%) with *E. granulosus*. The highest occurrence was observed in western region of Yazd and the lowest one was reported from the east region.

**Conclusion**: Our study showed the emphasizing of the importance of proper food safety measures. The awareness of the people in this region is necessary to control the diseases by *Toxocara* spp. *Echinococcus* spp. adherence to hygiene practices to mitigate parasitic infection risks.

**Keywords**: Vegetables, *Echinococcus granulosus*, *Toxocara* **Correspondence Email(s):** 

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Paleoparasitological investigations through utilizing advantage of new technical methods

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**Background**: Paleoparasitology is the study of parasites in the past. Nowadays, new methods have been developed to detect and analyze ancient parasites. Ancient parasites can be obtained from mummies, coprolites, latrines, burial soil, etc. Parasitic infections like malaria can cause anemia and leave traces on the skeleton.

**Methods:** Symptoms like cribra orbitalia, cribra cranii, and porotic hyperostosis can help identify skeletons suspected of having anemia. Then, we can use other methods, such as molecular methods, for further investigations and to confirm the presence of parasites. Due to advances in omics data generation, data analysis, and techniques like genomics, proteomics, etc., paleoparasitology has made significant strides.

**Results**: These new techniques have greatly facilitated identifying and analyzing parasites in ancient remains and opened new avenues for a better understanding of past health and disease

**Conclusion**: These techniques have enhanced our knowledge of human history's prevalence, impact, and evolution of parasitic diseases. By identifying ancient diseases, we can better understand the past, the obstacles our ancestors confronted, and how to overcome them in the future.

**Keywords**: Paleoparasitology, ancient parasites, genomics, proteomics

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Poster

Paromomycin-loaded mannosylated chitosan nanoparticles: targeted drug delivery against BALB/c mice infected To *L. major* 

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**Background**: Leishmaniasis is a disease with unsatisfactory current therapies due to the emergence ofdrug resistance and toxicity. Paromomycin (PM), suffers from poor oral bioavailability, limited efficacy and rapid clearance inparenteral route. In this study, we examined the efficacy of nanoparticle-based PM delivery system intreating the murine infected with *Leishmania major*.

**Methods:** Paromomycin was loaded inmannosylated chitosandextran nanoparticles (PM-MCS-dex-NPs) through ionic gelation method. The particle size and zeta potential of PM-MCS-dex-NPs were obtained as 246 nm and +31 mV, respectively. PM-MCS-dex-NPs effectively affected both stages of the parasite especially the amastigote one in vitro culture. Nanoformulation injected intramuscularly into mice for up to 21 days. Lesion sizes were measured before the onset of treatment and at weekly intervals for a month. In addition, the DNA copy number was quantified in the infected mice by a real time quantitative polymerase chain reaction (qPCR).

**Results**: In vivo results showed that the administration of PM-MCS-dex-NPs with a dose of 10 mg/kg/twice daily significantly reduced the lesion size and DNA copy number compared to the other treatment methods. Lesions sizes in both control groups of chitosan nanoparticles (CS-NPs) and mannosylated nanoparticles (MCS-NPs) were also significantly (P < 0.05) decreased in comparison with the untreated control, suggesting the wound healing property of chitosan.

**Conclusion**: PM-MCS-dex-NPs proved as a promising candidate in delivering PM by boosting the drug solubility and targeting the infected macrophage cells. The results of this study can provide a new and efficient drug delivery system for CL treatment.

Keywords: Paromomycin, mannosylation, qPCR, leishmaniasis, L. major Correspondence Email(s): motazedm@sums.ac.ir



Passive case findings on malaria in Yazd as a central province of Iran during 2011-2020

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**Background**: Malaria is considered one of the most serious life-threatening diseases in Yazd, a central province of Iran, which hosts both domestic and foreign immigrants. This study aimed to investigate the trend of malaria in Yazd during 2011-2020.

**Methods:** In this descriptive retrospective study, all episodes of the disease (imported malaria) recorded at Yazd health center in Iran were carefully evaluated and reported. After preparing the peripheral blood smear and fixation with methanol, it was stained with Giemsa and examined with a light microscope by a skilled technician.

**Results**: A total of 95 confirmed malaria patients were investigated from 2011 to 2020. *Plasmodium falciparum* was the predominant species with 81 cases (85.26%). The highest rate of infection was observed in 49 cases (51.63%) from Yazd city in the age group of 30-39 years with 29 cases (30.53%), and the working class with 69 cases (72.63%).

**Conclusion**: Despite a decrease in malaria cases through the implementation of the eradication program compared to the last two decades, its imported type is still showing up in the country, especially in the cosmopolitan city of Yazd, where domestic and foreign workers and tourists usually travel.

**Keywords**: Malaria, Yazd, prevalence, *Plasmodium falciparum* 

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Poster

Pathology, diagnosis and treatment of demodicosis

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**Background**: Demodicosis in humans caused by two species of Demodex called folliculorum and brevis. Dermatitis and rough, dry and scaly skin, particularly asymmetrical papulopustular or granulomatous variants are observed. Clinical symptoms of demodicosis mimic other known skin diseases such as dermatophytosis, fuliculitis, rosacea, perioral dermatitis and blepharitis.

**Methods:** Some of the physicians treat the patients according to the clinical symtomes. Scraping from the skin of lesions, Slide preparation with 20 % KOH and observing the mite under the microscopic is the gold standard diagnostic test. Histopatologic examination of skin biopsy can determine the presence of *Demodex*. The patients infested with Demodex folliculorum were referred to dermatologists for treatment.

**Results**: The patients include 248 (77.74%) female and 71 (22.26%) male at the age of 8 months to 81year (mean 35.6 years). Common interventions used for Demodex infestation include Benzyl benzoate, Crotamiton, Ivermectin, Metronidazole, Permethrin, Salicylic acid, Selenium sulfide, Sulfur products. 6 mg of ivermectin taken orally twice daily at 2-weeks intervals and 1% cream reduced the average number of *Demodex* mites in chronic *Demodex blepharitis*. Local and systemic corticosteroids are contraindicated in any patient diagnosed with demodicosis. Secondary bacterial infections must be treated aggressively with an appropriate antimicrobial.

**Conclusion**: The patients who were treated with systemic and local ivermectin had better healing. Before administration any treatment, the cause of disease must be clarified. Local and systemic corticosteroids are contraindicated in any patient diagnosed with demodicosis.

**Keywords**: *Demodex folliculorum*, demodicosis, diagnosis, treatment

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Performance of polymerase chain reaction for the detection of hydatid cyst using serum samples

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**Background**: Hydatid cyst caused by the larval stage of *Echinococcus granulosus* is an important zoonotic disease in various parts of Iran. This parasitic disease is usually diagnosed using imaging and serological techniques; however, several studies have recently suggested using the larval stages-derived DNAspecific polymerase chain reaction (PCR) on serum samples.

**Methods:** Among 400 patients with space-occupying lesions or cystic masses detected by ultrasonography or computerized tomography (CT) scan who were referred to the Parasitology and Mycology Department of Fasa University of Medical Sciences to detect antibodies against hydatid cyst, conventional PCRs targeting the *E. granulosus*-specific the cytochrome c oxidase 1 (cox1) and NADH dehydrogenase subunit 1 (nad1) mitochondrial genes were carried out on sera of 15 patients with intact hydatid cyst(s) who were histopathologically confirmed. DNA extracted from sheep hydatid cyst protoscolices was used as control positive.

**Results**: DNA of *E. granulosus* was not found to be excreted in serum samples.

**Conclusion**: Serum sample might not serve as useful samples for the molecular recovery and detection of hydatid cyst in patients.

Keywords: Hydatid cyst, serum, PCR, detection

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Phylogenetic and molecular study of *Nosoma* sp. isolated from adult bees in west Azerbaijan, Iran

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**Background**: *Nosema* is an opportunistic intracellular paratiste that belonging to Microsporida. This single cell is one of the most important in Honeybees. It is very difficult to identify species using microscopic method due to the similarity of morphological factors, so it is important to use other methods including molecular techniques.

**Methods:** In this study, 133 apiaries from the cities of West Azerbaijan provinces were investigated. For all the morphologically positive samples, the PCR reaction was performed using species-specific primers based on SSUrRNA gene.

**Results**: All the samples from the investigated areas are N.cerana.Sequence analysis showed 100% similarity and homology of all the samples. Comparing the sequences with bioinformatics method showed that there were no subspecies or haplotypes different in these regions with no intraspecific variation. The results showed that the samples in this province are in an one haplogroup network with different from the samples in the GenBbank.

**Conclusion**: It seems that the molecular distribution pattern of this protozoa is stable in west Azerbaijan and this is a good opportunity to control the disease among the apiaries of these regions.

**Keywords**: Phylogenetics, *Nosoma*, SSUrRNA, Azerbaijan

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Phylomolecular study of Fasciola spp. based on pepck and Cox1 markers isolated from Urmia and Maragheh, northwest Iran

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**Background**: Fascioliasis is a significant zoonotic disease caused by F. hepatica and F. gigantica. Species identification of Fasciola in different regions of Iran is necessary. The present study aimed determine the species and characterize haplotypes and phylogenetic patterns of Fasciola using morphometric, molecular, and bioinformatics techniques.

Methods: Ninty six liver specimens were collected from naturally infected sheep (Ovis sp.) and cattle (Bos taurus) in two geographical regions from 2021 to 2022. After fixation and morphological studies, DNA was extracted from each flukes using commercial kits according to instructions. Then pepck and cox1 genes were amplified using specific primers. For discrimination of Fasciola species, Multiplex-PCR on pepck was used. Phylogenic and genetic analyses were done based on cox1 sequence using Maximum Likelihood, MegaX, sequencher 4.1, Dnasp5, and popart softwares.

**Results**: Out of 96 *Fasciola* specimens, we obtained 33 *F*. gigantica and 63 F. hepatica. Cox1 gene sequences were deposited in GenBank. Phylogenetic tree and haplotype diversity indices showed that there are different populations of F. hepatica in Urmia and Maragheh, northwest Iran.

**Conclusion**: There is no intermediate form of *Fasciola* sp. High haplotype and nucleotide diversity could be due to ecological factors in life cycle, animal migration, and coexistence of the final host of the parasite. Haplotype and nucleotide diversity of F. hepatica in Iran and this study showed variety of haplogroups.

Keywords: Fasciola, pepck, Cox1, northwest, Iran treatment Correspondence Email(s): saberraeghi@gmail.com

Poster

Pilot evaluation of treatment of cutaneous leishmaniasis with thymol loaded on chitosan gel, as a complementary topical treatment

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**Background**: Leishmaniasis is a parasitic disease, but no effective drug has been found yet. Pentavalent antimony compounds are toxic and in some cases ineffective and treatment with them is long. Therefore, The effect of thymol in combination with chitosan has been evaluated to treat the Leishmania wounds.

Methods: First, chitosan gel containing 5 and 10% thymol was prepared. Microbial control, drug release and XRD, FTIR and DSC tests were performed on the synthesized product. For clinical studies, patients with leishmaniasis lesions were randomly divided into four treatment groups. Patients' follow-up was performed for 60 days and wound size was measured. In the four treatment groups, a standard group measured and compared the effect of the intervention. In each group, the size of the wound (diameter or area) was determined by photographing the wound using imag J software.

**Results**: The results showed that the rate of thymol release from the chitosan gel reached the plateau level after about 6 hours (with a slow upward trend for both samples). Due to the fact that in the DSC results, the melting temperature of thymol has changed from a sharp peak to a wide peak and shifted slightly to the left, it seems that among the chitosan fibers in the hydrogel structure, there is more physical stability for thymol. Also, the groups receiving standard treatment with chitosan 2% containing thymol 5 and 10% experienced a much more effective recovery process.

**Conclusion**: Obtained results show that the use of thymol loaded in chitosan gel can effectively improve the lesions caused by skin leishmaniasis with standard treatment.

Keywords: Leishmaniasis, thymol, chitosan, topical

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Polymer nano-capsules of Albendazole and Mebendazole for the treatment of hydatid cyst infection: in vivo efficacy studies

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**Background**: Nano-drugs offer potential solutions for treating hydatidosis, a parasitic infection. Their small size allows for targeted delivery, enhancing efficacy while minimizing side effects. Despite challenges like toxicity, ongoing research aims to optimize their safety and effectiveness. Nano-formulated medications hold promise in combating hydatid cysts, offering hope for improved treatment outcomes.

**Methods:** Polymeric drug-loaded Nano-capsules were synthesized using a micro emulsion of oil in water. Formulations were assessed for droplet size, zeta potential, and drug loading/release. After seven months of infection, 56 hydatidosis-infected mice were divided into seven groups. Groups 1, 2, and 3 were treated daily with Albendazole Polymer Nano-capsules (Nano-ABZ), Mebendazole Polymer Nano-capsules (Nano-ABZ), and Albendazole + Mebendazole Polymer Nano-capsules (Nano-ABZ + MBZ) for two months. Groups 4, 5, and 6 were treated with Albendazole (ABZ), Mebendazole (MBZ), and Albendazole + Mebendazole (ABZ), while group 7 served as the negative control group without treatments (only received PBS).

**Results**: According to the results, the Nano-ABZ + MBZ group had significantly lower total cyst numbers, total cyst weight, and maximum cyst size compared to the Nano-ABZ and Nano-MBZ groups. Additionally, all three Nano-capsule groups had significantly lower total cyst numbers compared to Non-Nanocapsule groups and the negative control group.

**Conclusion**: In conclusion, ABZ, MBZ, and ABZ + MBZ Nano-capsules can serve as suitable alternatives to chemical drugs. With the appropriate application of nanotechnology, it is possible to develop safe and effective drugs, such as the polymeric combination of Albendazole and Mebendazole, which holds promising implications.

**Keywords**: Polymer Nano-capsules, hydatidosis

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Preparation and characterization of paromomycin encapsulated in lecithin-chitosan nanoparticles for the topical treatment of cutaneous leishmaniasis

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**Background**: Leishmaniasis is a disease caused by the *Leishmania* protozoa, with cutaneous leishmaniasis (CL) being the most common form of the illness. Paromomycin (PM) has recently garnered increased interest for its effectiveness against *Leishmania*, but it is hindered by limited efficacy, low oral bioavailability, and rapid clearance.

**Methods:** The loading of PM into lecithin-chitosan nanoparticles was achieved using the ionic gelation method, and the resulting nanoparticles were characterized. The IC50 values for PM, Glucantim, and Nano-PM against promastigotes were determined after 24, 48, and 72 hours treatment. The viability of promastigotes was assessed using the MTT assay. The Nano-PM formulation was administered intramuscularly to mice for a period of 28 days, during which lesion sizes were measured weekly. Furthermore, the parasite load in the infected mice was quantified using quantitative real-time polymerase chain reaction (qPCR).

**Results**: IC50 of Nano-PM was significantly lower than Glucantim (P = 0.0001 after 24 h incubation, P = 0.013 for 48 h and P = 0.0001 for 72 h) and PM (P = 0.0001 after 24 h, P = 0.003 for 48 h and P = 0.0001 for 72 h). All concentrations of Nano-PM had the highest toxicity on promastigotes in comparison with other groups after 24, 48 and 72 h treatment. Moreover, a significant reduction in the lesion size was found in the Nano-PM group in comparison with the control group after three (P = 0.0369) and four (P = 0.0009) weeks treatment. More importantly, Nano-PM significantly reduced the parasite load compared to the control and the lecithin-chitosan groups (P = 0.001 for both).

**Conclusion**: Our findings showed that Nano-PM had lower toxicity (lower IC50) on promastigotes compared to glucantim and PM. Moreover, Nano-PM treated mice showed reduced lesion size compared to the control group. Additionally, Nano-PM led to a significant decrease in parasite burden compared to the control group and the lecithin-chitosan group.

Keywords: Leishmania major, paromomycin, lecithins, chitosan

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## Poster

Prevalence and factors related to trichomoniasis, *Candida* vaginitis and bacterial vaginosis

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**Background**: Candida species, *Trichomonas vaginalis* and bacteria are considered to be the main causes of vaginitis. This study investigated the prevalence of *Candida*, *Trichomonas* and bacterial vaginitis and factors related to infection in women.

**Methods:** This cross-sectional study was conducted to investigate the relationship between different individual characteristics and common vaginal infections namely candidiasis, trichomoniasis, and bacterial vaginosis. The sample included all women referred to Allameh Bohlool Gonabadi Hospital women's clinic in Gonabad in 2021. After the patient's physical examination and questionnaire completion, samples were taken from the mucous secretions of the cervical vagina and the posterior fornix region using three sterile swabs. The first swab of secretions was placed on three glass slides for microscopic examination. At the same time, the second swab was transferred to the special *Trichomonas* culture medium (Dorset culture medium).

**Results**: The prevalence of candida, trichomonas and bacterial vaginitis in the admitting women was 5%, 38.5% and 5.8%, respectively. A significant relationship was found between the history of vaginal infection, trichomonad infection, and candida infection (P = 0.03). Moreover, significant relationships were observed between bacterial infection and the husband's occupation (P = 0.002), methods of preventing pregnancy (P = 0.01) and menopause (P = 0.001).

**Conclusion**: Vaginal infections are one of the common problems in women of all ages, and by knowing the factors that cause these infections, a big step can be taken to reduce the problem.

**Keywords**: Vaginitis, candidiasis, bacterial vaginitis, *Trichomonas* 

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## Poster

# Prevalence and genetic characterization of Cryptosporidium in pre-weaned cattle in Urmia (northwestern Iran)

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**Background**: Cryptosporidiosis is a zoonotic disease causing digestive problems in pre-weaned calves. Considering the zoonosis of the parasite and its importance in veterinary medicine, we evaluated the prevalence and genotyping of *Cryptosporidium* spp. in diarrheic pre-weaned calves in the northwest of Iran.

**Methods:** A total of 100 stool samples of the infant calves with diarrhea were collected from industrial and conventional livestock farms in Urmia City. All the samples were tested with acid-fast staining, ELISA, and PCR. Positive samples of the PCR method were sequenced to determine the *Cryptosporidium* species. The obtained results were compared for the mentioned methods based on statistical factors, sensitivity, specificity, positive and negative predictive values, as well as duration of the experiment and the costs of testing.

**Results**: The results of this study showed that the prevalence of *Cryptosporidium* spp. in diarrheic infant calves in Urmia city was 5%, and *C. parvum* species of *Cryptosporidium* was detected in all the sequenced samples. According to the findings of the current study, the most appropriate method for the detection of the parasite is the ELISA that has a higher sensitivity and predictive value than acid-fast staining method and should be used in veterinary laboratories.

**Conclusion**: In the current investigation, *C. parvum* was identified as the only infectious agent in the region and could be the main cause of human infection. More studies are needed to find the source of infection for establishing the control measures.

**Keywords**: *Cryptosporidium*, prevalence, genetic characterization, sensitivity

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#### Poster

Prevalence and genotypic distribution of *Trichomonas* vaginalis in women referred to a women's clinic in Mashhad

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**Background**: *Trichomonas vaginalis* is a common sexually transmitted parasite that can cause vaginal infections in women. In this study, we aimed to investigate the prevalence and genotypic distribution of *T. vaginalis* along with demographic and behavioral factors as well as the clinical manifestations in women referred to women's clinic.

**Methods:** Vaginal soap samples were collected from the 80 participants, and both microscopic and molecular tests were performed to detect the presence of *T. vaginalis*. In addition, demographic and behavioral information was collected and analyzed. Nested PCR and sequencing were conducted for the Actin gene to determine the genotypes of *T. vaginalis* in the positive samples.

**Results**: Out of the 80 samples analyzed, 17 were found to be positive for *T. vaginalis*. Sequencing analysis revealed that 3 samples belonged to genotype G, while 9 samples belonged to genotype H. Notably, none of the positive women were in the menopausal period, and a significant proportion of them were pregnant. Furthermore, only 3 of the positive women had diploma and higher education, and a majority of them were housewives. The main clinical signs observed in these patients included burning, itching, redness, and inflammation.

**Conclusion**: The study revealed high prevalence and genetic diversity of *T. vaginalis* in Mashhad, with genotypes G and H identified. Associations with pregnancy and lower education levels were noted, highlighting potential risk factors. Clinical signs emphasized the importance of early diagnosis and treatment.

Keywords: Trichomonas vaginalis, genotype, prevalence

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## Poster

Prevalence and molecular analysis of Cryptosporidium spp. recovered from surface waters

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**Background**: *Cryptosporidium* is an obligate intracellular parasite reported from all over the world. This protozoan infects a wide range of animals. About 20 species of this parasite have been reported in humans, but *Cryptosporidium parvum* and *Cryptosporidium hominis* are the most prevalent infecting species in humans.

**Methods:** In this study, 42 samples were collected from 14 rivers in the catchment area of Lake Urmia. Moreover, Amplification of SSU rRNA gene was performed, and polymerase chain reaction products were sequenced.

**Results**: The results of sequencing and comparison of the sequences with the GenBank revealed that all the 17 positive samples were *C. parvum*, a zoonotic species and one of the most frequent human-infecting species.

**Conclusion**: Considering these data, it is highly important to prevent the spread of this protozoan by treating livestock and preventing human and animal effluents from entering the water.

**Keywords**: *Cryptosporidium*, prevalence, molecular analysis, surface

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Prevalence and risk factors of *Strongyloides stercoralis* infection in Babol Medical Centers, Iran

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**Background**: Strongyloidiasis is a persistent human parasitic infection caused by the intestinal nematode, *Strongyloides stercoralis*. Infection usually remains asymptomatic, but it could cause hyperinfection and dissemination in immunosuppressed patients, especially under corticoid therapy and would result in a high mortality rate.

**Methods:** The study was conducted for a period from December 2022 to December 2023. Demographic data (e.g., age, sex, place of resident) were obtained from the main registry in Babol city and suburbs. Fresh fecal samples were collected from 3827 patients and examined. Microscopic examination was performed using direct techniques (saline and iodine wet mounts).

**Results**: Only 38 cases out of 3827 tested samples, found positive with *S. stercoralis* infection. 21 (55%) of patients were from rural area and 17 (45%) were from urban areas. Also, the study population included 6 (15.7%) cancer cases and 8 (21%) of patients were treated by corticosteroids. The prevalence of strongyloidiasis infection was estimated to be 0.98 %. The prevalence of strongyloidiasis was increasing with the increase of age remarkably there was an increase in the age group 41-80 years of age (79.2%). The prevalence rate of parasites in males and females were 53% (n = 20) and 47% (n = 18), respectively.

**Conclusion**: Since disseminated strongyloidiasis is fatal in 80% of its cases, it is recommended to diagnose and treat the asymptomatic infection of this parasite before corticotherapy. Consequently, all physicians develop especially regarding patients with unspecific gastrointestinal symptoms, before chemotherapy or steroid therapy to rule out the presence of larvae.

Keywords: Corticosteroid therapy, immunosuppression, parasite

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Poster

Prevalence and risk factors of *Toxoplasma gondii* among women with miscarriage and their aborted fetuses in northwest of Iran, 2020-2021

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**Background**: Toxoplasmosis is a worldwide infection of wide variety of animals and human. Abortion and congentital defects are amongst important risks of the infection. The aim of the present study was to determine the *Toxoplasma* caused abortions among women with miscariage in Urmia, northwest of Iran, 2020.

**Methods:** This cross-sectional study was conducted with 215 women with abortion and their aborted fetuses, from 2020 to 2021. Seroprevalence of anti-*Toxoplasma* IgG and IgM were determined using the sera of the aforesaid women. Nested PCR was carried out using RE-529 gene sequences, and sequencing was performed using the *Toxoplasma gondii* GRA6 gene on the remnant of pregnancy after abortion. The tissue positive samples were then subjected to another PCR on GRA6 gene and sequenced for genotyping.

**Results**: Out of 215 serum samples of women with abortion, 70 (32.6%) were positive and 145 (67.4%) were negative for anti-*Toxoplsma* IgG, and also three (1.4%) were positive for IgM. The RE-529 sequence of *T. gondii* was positive in three (1.4%) of the aborted fetuses. All three positive samples belonged to the *T. gondii* Type I genotype using GRA6 gene.

**Conclusion**: Our findings suggest that *T. gondii* is one of the causative agents of spontaneous abortion in west Azerbijan province, the northwest of Iran.

**Keywords**: *Toxoplasma gondii*, abortion, congenital toxoplasmosis

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Prevalence and subtype distribution of *Blastocystis* in individuals attending health centers in Torbate Heydarieh district

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**Background**: *Blastocystis* is a common protozoan parasite found in the gastrointestinal tracts of humans and animals. In this study, we aimed to investigate the prevalence and subtype distribution of Blastocystis, demographic data and risk factors associated with *Blastocystis* infection, in individuals attending health centers in the Torbate Heydarieh district.

**Methods:** A total of 203 stool samples were collected from individuals reffered to health centers in the Torbate Heydarieh district. Microscopic examination, culture, and PCR testing were performed to detect the presence of *Blastocystis*. Subsequently, sequencing was carried out for subtype determination. Demographic data and risk factors, including education level, occupation, animal contact, personal hygiene, and vegetable consumption habits, were evaluated.

**Results**: Out of the 203 participants, 20 males and 7 females tested positive for *Blastocystis*. However, none of the risk factors evaluated showed a significant difference between infected and non-infected individuals. The majority of infected individuals were asymptomatic, and no significant association was found between infection and the presence of symptoms. The occupational group with the highest number of positive cases was ranchers, with 9 positive cases out of 60 individuals in this category. Subtype 3 was the most common subtype, with 12 cases identified, while subtypes 1, 2, 5, and 7 were also detected.

**Conclusion**: The study found a high prevalence of *Blastocystis* infection in the Torbate Heydarieh district, with subtype 3 being the most common. Despite evaluating various risk factors, no significant associations were found, highlighting the need for further research to understand the transmission dynamics of this parasite.

Keywords: Blastocystis, subtype, Torbat Heydarieh, PCR Correspondence Email(s): salehisgh@mums.ac.ir Poster

Prevalence of *Blastocystis* in patients referred to Bushehr medical centers and its relationship with urticaria

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**Background**: Recent studies determined that the amoeboid form of *Blastocystis* acts as a factor in stimulating the host's immune responses and ultimately results in urticaria and other skin disorders. The present study was conducted in order to determine the prevalence of *Blastocystis* in people referred to Bushehr city health centers.

**Methods:** Fecal samples were collected from 180 males and females referred to Bushehr health centers and a questionnaire containing demographic information was completed for each person. Samples were examined by preparing direct smear (wet mount) and then formalin-detergent sedimentation techniques. Data were analyzed using SPSS 22.0 software and *Chi square* test.

**Results**: The results showed that 11.1% of cases infected with *Blastocystis* and 55% of patients with blastocystosis had various gastrointestinal symptoms. Statistical analysis showed that there was no significant relationship between infection with some demographic factors such as sex, age, literacy level and residence, but this was significant with some clinical symptoms such as itching and urticaria.

**Conclusion**: Despite the existence of conflicting information and many ambiguities about the Blastocystis, this emerging pathogen is very important in terms of causing allergic and skin disorders in sufferers, therefore, it is necessary that patients with urticaria be evaluated for blastocystosis along with other diagnostic procedures and physicians.

Keywords: Prevalence, Blastocystis, urticaria, Bushehr, Iran

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Prevalence of Cryptosporidium parasite in children of Larestan in 2020

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Background: Cryptosporidium parasite is from Coccidian group that causes digestive diseases in people who have a weak security system or suffer from AIDS. This parasite has no special host. Although, the infection is usually stopped spontaneously in normal individuals, the quality of self-pollution and extension of this parasite is possible to diagnostic.

Methods: In this research, we collected 725 samples of fects from eight area of south of Iran. 45 samples were watery as having diarrhoea. We used the colour method (Ziehl-Neelsen's modified by Hendrickson) for diagnosis.

**Results**: To colour these samples did not show any sign of pollution with Cryptosporidium in these children. It was probably because either the facet samples were not sufficient or at the time of survey (Autumn and Winter), the rate of pollution had been less.

**Conclusion**: It is nevertheless a require to continue carefully this research and to find its prevalence which is a real danger for the infants' health and security.

Keywords: Cryptosporidium, children, Iran

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Poster

May 21 - 23, 2024

Prevalence of intestinal parasites in food handlers and study of genetic diversity of Blastocystis spp. isolated from Birjand in 2022

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**Background**: The aim of study was to investigate the prevalence of intestinal parasites in Food handlers and genetic diversity of *Blastocystis* spp. subtypes in Birjand.

Methods: In a cross-sectional study, 1020 stool samples were randomly collected from January to December 2022 in Birjand Health Center Laboratory. The Clinic visitor's demographic details were recorded. Samples were examined by a routine direct fecal examination, formalin-ethyl acetate sedimentation concentrate and trichrome staining as complementary techniques. Genomic DNA was extracted and PCR performed on *Blastocystis* spp. positive samples.

**Results**: The prevalence of parasitic organisms was 16.7% in the food-handlers. The most species of the protozoan parasites were: Eentamoeba coli (36.5%), Blastocystis hominis (30.1%), Giardia lamblia (17.4%), Chilomastix mesnili (12%), Trichomonas hominis (1.8%) and Entamoeba histolytica (0.6%); meanwhile, only 3 case infected by Hymenolepis nana (1.8%) was detected in this group. There was no significant statistical difference in the rate of infection among age and occupation groups. A significant relationship showed between parasitic infections and participation in the health-training course ( $P \le 0.05$ ) and education level ( $P \leq 0.05$ ). The results of the sequencing showed the dominance of ST3 subtype (68%) and ST2 (32%).

Conclusion: The high frequency of protozoan parasite in this study again highlights the hazard of transmission by food handlers. Teaching the science of parasitology in simple language in training classes for food sellers will play a significant role in reducing the spread of parasitic diseases.

Keywords: Intestinal parasites, pathogenic protozoa, PCR,

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Alborz University of Medical, Sciences, Karaj, Iran May 21 - 23, 2024

5th International & 12th National Congress of Parasitology and Parasitic Diseases of Irai

Poster

Prevalence of intestinal parasitic infections among labor children in Karaj, Iran.

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**Background**: Enteric helminthic and protozoal infections are prevalent especially among children with weak health conditions. Child labor is a serious national and international humanity/ health issue, correlated with socioeconomic conditions. The aim of the present study was to evaluate the prevalence of intestinal parasites in child labor, in Karaj city.

**Methods:** In present cross-sectional study, stool samples from 203 labor child have been collected and examined by using concentration and parasitology staining methods, like Merthiolate-iodine-formaldehyde, Trichorom, Acid fast and Hot gram chromotrope 2R. Demographic, socio-economic factors, education level, and their parent's information collected by using a questionnaire.

**Results**: The overall prevalence of intestinal parasites in children was estimated at 76.9%. The frequently encountered infections included *Entamoeba histolytica*/dispar (3.9%), *Giardia lamblia* (5.4%), *Entamoeba coli* (24.1%) and *Hymenolepis nana* (0.5%). There were no statistically differences among the sex, age and family size. The level of parents' education represents a risk factor of infection for the children (P < 0.05).

**Conclusion**: Considering the high prevalence of intestinal parasitic infection among labor children according to the present study, as well as, the low level of health and nutrition in them and the low level of parents' education, a codified and interdisciplinary program with the presence of the organization is suggested.

**Keywords**: Intestinal parasite, infection, labor children, riskfactor

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Prevalence of intestinal protozoan infection in patients with ulcerative colitis (UC) in Isfahan, Iran

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**Background**: Determination of the prevalence of intestinal protozoan infection is a fundamental step to set up an effective control program to improve the health status of society and to establish efficient strategies. Intestinal pathogen and even non-pathogen protozoa consider as major causes of disease in patients with gastrointestinal problems.

**Methods:** The descriptive cross-sectional study carried out from 2013 to 2018 in Isfahan, Iran. One thousand nine hundred and sixty-five samples of feces from patients with UC collected and each sample examined using direct wet mounting with normal saline and iodine and sedimentation tests such as formol-ethyl acetate concentration and trichrome-staining methods.

**Results**: From 655 patients, 185 (28.2%) infected with Giardia lamblia followed by *Blastocystis hominis* (27.3%), *Endolimax nana* (14.4%), *Entamoeba coli* (11.5%), *Iodamoba butschlii* (4.7%), *Entamoeba histolytica* (1.4%), and *Chilomastix mesnili* (0.6%).

**Conclusion**: This study revealed a high prevalence of infection with at least one or six non-pathogenic and pathogenic intestinal protozoa in UC patients in the Isfahan region. Intestinal protozoa are a challenging public health problem wherever health care is limited in the area.

Keywords: Blastocystis hominis, Giardia lamblia,

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Prevalence of leishmaniasis in Isfahan province during 1394 to 1399

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**Background**: The basis of proper care for each disease is to determine the definitions of the disease and their timely registration and reporting.the aim of this study was to determine the prevalence of Leishmaniasis in Isfahan province during the years 1394 to 1399

Methods: This descriptive cross-sectional study was conducted in 1400 in Isfahan province. The study sample was equal to 11589 patients with leishmaniasis in the last 6 years (1394 to 1399) who were selected by census sampling and entered the study. The data were described and analyzed by SPSS software at a significance level of less than 0.05.

Results: According to the results of this study, the highest number of seeker referrals in 1397 was 5.6 people per 10,000 population. The prevalence of leishmaniasis in women and in Isfahan province is equal to 17 per 10,000 population of women and equal to 28 in men. The highest rate of leishmaniasis per 10,000 population is in Natanz city with 144 people and the city in Isfahan was equal to 39 people. The highest frequency of seekers in women was in Natanz city with 114 people per 10 thousand population and the highest frequency of seekers in men was in Natanz.

Conclusion: using the obtained result the necessary strategies and measures can be implemented to reduce leishmaniasis, prevent the spread and provide public health. Also according to the distribution plan of the province, the necessary strategies should be taken to improve the situation of the infected areas and preventive measures should be taken.

Keywords: Leishmaniasis, epidemiology, Isfahan province

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May 21 - 23, 2024

Prevalence of parasitic contamination of raw vegetables in Shiraz County, southern Iran in 2022

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**Background**: Vegetables play an important role in the transmission of parasitic infections. Considering the existence of endemic parasitic diseases in Iran and the role of infected vegetables in the transmission of these infections, the purpose of this study is to investigate the parasitic contamination of raw vegetables consumed in Shiraz county.

Methods: In this study, 6 types of raw vegetables, were collected randomly from 50 vegetable shops of different areas in Shiraz and Sadra city. The vegetables were washed with anionic detergent and then centrifuged. The sediment was checked for parasitic contamination by direct methods, Lugol staining and sucrose flotation.

**Results:** Parasitic contamination was observed in 10 vegetable shops (20%). In 8 vegetable shops, the vegetables were infected with free-living larvae (16%), the vegetables of one shop were infected with Trichostrongylus eggs (2%), and one vegetable shop infected with free-living larvae was and Trichostrongylus eggs (2%) at the same time. All 10 cases were related to vegetable shops in Shiraz city.

**Conclusion**: The contamination of vegetables with pathogenic and non-pathogenic parasites is relatively high in Shiraz county. Most of the positive cases in this study are not pathogenic for humans, but they are considered as food health indicators.

Keywords: Parasitic infection, Vegetables, Shiraz, Iran

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Prevalence of parasitic infections in cancer patients and healthy individuals in cancer patients and Healthy individuals in Isfahan, Iran during a 5-Year investigation

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**Background**: The objective of this study is to evaluate the prevalence of parasitic infections in cancer patients and control group in Isfahan, Iran (2014-2019).

**Methods:** Three Stool samples from 187 cancer patients and 144 healthy individual collected. Direct smear using the normal saline, Lugol's iodine staining, Formalin-ether concentration method, Modified Ziehl-Neelsen acid-fast method, and modified Trichrome staining technique performed for each sample.

**Results**: Generally, the prevalence of parasitic infection in cancer patients was 39%. The rate of infection in control group was 28%. *Blastocystis* hominis was the most prevalent parasite in both cancer patient and control group, 18.7% and 13.2%, respectively. Other parasitic infection were as follows: *Entamoeba coli* (10.2%), *Endolimax nana* (6.4%), and *Giardia lamblia* (4.8%)

**Conclusion**: Prevalence rate of parasitic infections particularly helminthic cases declined during the past decades. However, protozoan parasitic infections are still considerable.

Keywords: Parasitic infection, Iran, cancer patients

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Poster

Prevalence of theileriosis in industrial dairies of Buin-Zahra City

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**Background**: This study was performed to evaluate bovine theileriosis prevalence in dairy cattle in the summer, in Buin-Zahra city of Qazvin province.

**Methods:** After recording the details of each farm and numbers of selected livestock in the questionnaire, clinical examination of all the animals were taken and blood smears were prepared and sent to the laboratory. The samples after fixation with methanol and stained with Giemsa's Azur-Eosin were investigated for the presence of *Theileria* spp.

**Results**: The results of this study revealed that in a total of 350 dairy cattle, 11(3.14%) cattle were infected with *Theileria* spp., also clinical symptoms include, pale mucous in 5 cases (45.4 %), swelling of lymph nodes in 8 patients (72.7%), jaundice in 3 cases (27.2 %), bleeding point mucosa in 3 cases (27.2 %), diarrhea in 2 cases (18.1%) and cough in 1 (9%) were observed. The highest infection rates among different age groups of less than one year (54.6%) and lowest infection rate of those aged over 3 years (9 %).

**Conclusion**: The highest infection rates among different age groups of less than one year (54.6%) and lowest infection rate of those aged over 3 years (9%).

Keywords: Theileriosis, cattle, Buin-Zahra

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Prevalence of *Toxoplasma gondii* in pregnant women referred to a health center in Qazvin province, Iran

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**Background**: Prevalence of *T. gondii* in pregnant women can be a criteria for risk of intrauterine transmission of the parasite in each region. The present study was performed in order to determine the prevalence of *T. gondii* among pregnant women referred to a health care centers in Qazvin province, Iran.

**Methods:** A total of 368 pregnant women referred to a health centers in Qazvin province of Iran participated in this study in 2019. Sera of the subjects were examined for IgG and IgM antibodies to *T. gondii* by ELISA. IgG avidity test was used as a confirmatory test for samples which were positive for both IgG and IgM antibodies. Demographic data and knowledge of participants about *Toxoplasma* and toxoplasmosis were collected using questionnaires. The data were analyzed by using SPSS, Version 23. *Chi-Square* and ANOVA tests used to determine a significant association between *T. gondii* infection and variables.

**Results**: From 368 pregnant women, 26.1% (n = 96) and 0.54% (n = 2) were positive for *T. gondii* IgG and IgM antibodies, respectively. Index of IgG avidity test of the 2 cases who were IgM positive showed a high avidity. Only 1.9% (7/368) of the women had prior information about *Toxoplasma* and toxoplasmosis. Among variables, contact with soil was the only cause of a significant association with *T. gondii* infections.

**Conclusion:** We conclude that the prevalence of *T. gondii* in the study area is moderate level, but its incidence show remarkably reduction, which indicates a low risk of congenital toxoplasmosis in this region.

Keywords: Toxoplasma gondii, prevalence, pregnant women

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Prevalence of toxoplasmosis in patients infected with tuberculosis; a sero-molecular case-control study in northwest Iran, along with evaluation in laboratory animal

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**Background**: Tuberculosis (TB) and toxoplasmosis are emerging intracellular infectious diseases; and their co-infections in immunocompromised patients are one of the most serious health concerns all over the world, particularly in developing countries. In this study, we investigated the possible association between TB and *Toxoplasma gondii* infection.

**Methods:** One hundred confirmed TB individuals living in northwest Iran were classified into three subgroups; newly diagnosed patients (NTB), old diagnosed patients (OTB) and multidrug resistance patients (MDR-TB). One hundred healthy subjects in the same age and sex distribution were ethnically matched. Sera samples were screened for anti-*Toxoplasma* antibodies. Nested-PCR was performed by targeting the B1 and GRA6 genes. All positive samples of the molecular method were inoculated in laboratory mice to identify *T. gondii* tachyzoites. The laboratory animals used were inbred mice, all of which were male, approximately 60 days old, and approximately 24-27 grams in weight, which were divided.

**Results**: The frequency of *Toxoplasma* infection based on IgG titer was 71.1% in the OTB subgroup and 33% in the control group, indicating significant association between *Toxoplasma* seropositivity and OTB (P = 0.001). According to phylogenetic network, the type I strain of *Toxoplasma* was identified in the OTB subgroup (10.1%). Seven isolates were successfully amplified by targeting B1 and GRA6 markers in the OTB subgroup and only three sera samples were positive in high titer of IgG (200 IU/ mL). Buffycoat of 7 positive isolates were inoculated into inbred mice, but no increase in IgG and IgM antibody titer was detected.

**Conclusion**: Our findings showed a high seroprevalence of anti-*Toxoplasma* IgG antibodies in the OTB subgroup (71.1%) compared to the control group (33%), revealing a meaningful association between *Toxoplasma* seropositivity and OTB patients and OTB subgroup are at high risk for acquisition of *Toxoplasma* infection which could reactivate the latent toxoplasmosis.

Keywords: Case-control study, Iran, toxoplasmosis, tuberculosis Correspondence Email(s):

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Prevalence of *Trichomonas vaginalis* infection in infertile women referred to Yasuj genycology clinic in 2019

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**Background**: Trichomoniasis is one of the parasitic diseases caused by the protozoan flagella Trichomonas vaginalis. It is transmitted through sexual contact and is one of the most common non-viral sexually transmitted diseases that can cause miscarriages, infertility, vaginitis and other diseases.

**Methods:** Out of 200 infertile women referring to Yasuj Gynecology Clinic and 153 entered in the study, they were satisfied to participate in the study. Using a sterile cotton swab from the posterior part of the vaginal fornix, the amount of secretions was collected. The specimens were examined by direct method in less than half an hour. Finally, the results were analyzed by SPSS software version 23 using T-test.

**Results**: Of the 153 vaginal samples, 13 (8.49%) were infected with *Trichomonas vaginalis* by direct observation. In the age group of 15 to 25 years (11.42%) and according to education, Those who have Diploma and associate the highest level of infection was observed. 13.3% of the patients with dysuria and 20% of the patients with itching were infected with Trichomonas (13.84%). Also, 17/64% of patients with symptoms of watery discharge, 6/25% of people with dyspareunia and 14/70% of people with more than two symptoms were infected with Trichomonas. Based on infertility types, the cause of ovarian factor 76 (7.89%).

**Conclusion**: According to the results of this study, one of the possible causes of infertility in women is *T. vaginalis* infection. Therefore, microscopic examination to detect trichomoniasis as one of the first steps in the treatment of infertile women seems to be reasonable.

Keywords: Trichomonas vaginalis, outbreak, infertile women

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Poster

Prevalence of trichomoniasis, and candidiasis among Iranian women with vaginitis

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**Background**: Trichomoniasis is the most common protozoal venereal disease and the second cause of sexual infections. Vaginal candidiasis is another infection affecting women. In recent years, the prevalence of candidal vaginitis has increased. In this study, the prevalence of *Candida albicans* and *Trichomonas vaginalis* co-infection among women was determined.

**Methods:** The descriptive survey was performed on women in women's clinic in Damavand city, Iran, from June 2023 to January 2024. The women were aged 30 to 70 years old. One hundred and twenty women suspected of vaginitis, participated in this survey. Before collection, the participants were completed a questionnaire. Three cotton swabs were used for collecting the samples. One swab was used for wet mount microscopy for parasite. Two swabs were used for yeast direct smear and culture in Sabouraud dextrose agar.

**Results**: From one hundred and twenty participants, a total of 52 (43.3%) participants had abnormal discharge. The highest frequency in age group of patients was 40 to 49 years old that this was statistically significant (P < 0.01). Vaginal irritation and pruritus (60.3%) were the most dominant signs in the patients. The association between infection with *T. vaginalis* and certain contraception methods was statistically significant (P < 0.01). *T. vaginalis* had the least prevalence (n = 6; 5.0%) while *C. albicans* had the highest prevalence (n = 46; 38.3%). In addition, 7 (13.4%) patients showed coinfection with *C. albicans* and *T. vaginalis*.

**Conclusion**: The two most infections of the genital system in women are trichomoniasis and candidiasis. Given the epidemiological profiles, physicians must be attention to recommend appropriate therapies. So, clinical and laboratory results are need. Results will help to increase knowledges about prevalence of vaginal infections in the Damavand city of Iran.

Keywords: Trichomonas vaginalis, Candida albicans, women

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ith International & 12th National Congress of Parasitology and Parasitic Diseases of Iran Alborz University of Medical, Sciences, Karaj, Iran

#### Poster

Prevalence survey of intestinal parasites in malignant patients in Omid Hospital of Urmia and isolation with molecular identification intestinal Microsporidia sp. in during 2019-2020

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**Background**: Protozoan infections are considered one of the most important causes of diarrheal disease in various malignancies. Microsporidia are among the common causes of asymptomatic, or self-limiting infections. This study investigated the prevalence of intestinal protozoa in malignant patients of Omid-Hospital, isolation with molecular identification microsporidia during 2019-2020.

**Methods:** 206 human stool samples were collected from patients who were referred to Omid Urmia Hospital due to any type of malignancy during 2019-2020. After collecting the demographic information, a macroscopic examination of the samples was done and a different staining such as Trichrome, Giemsa, Zil-Nelson, and wet smear were done. After extracting the DNA of the positive samples, using the sequence of the primers of the central region of the SSUrRNA gene of the Microsporidia genus and using the PCR-RFLP methods using the PstI enzyme and Nested-PCR, its species were detected in the positive samples.

**Results**: Out of the 206 samples, 67 people (32.5%) were infected with at least one type of pathogenic protozoa or non-pathogen, and only 1 case (0.48%) had worm infection. Protozoan infection was significantly higher than helminth infections (P = 0.001). The predominant intestinal pathogenic parasites were *Blastocystis* in 42 positive samples (19.9%), followed by *G. lamblia* in 8 cases (3.9%). The prevalence of different species of microsporidia was also found in 6 cases (2.9%). Out of 6 positive samples, 5 were positive for *Microsporidium* sp. in the microscopic method. Finally, the PCR-RFLP pattern showed that the species of microsporidia is Enterocytozoon bieneusi.

**Conclusion**: Diagnosis of microsporidiosis in different stages of the disease is imprtent. The use of a specific diagnostic method and course its identification in malignant patients will be doubly important. Therefore, it is important to use and launch methods that can detect infection in specific patients with malignancy.

Keywords: Intestinal protozoa, *Microsporidium*, northwest, malignancy

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### Poster

May 21 - 23, 2024

Protein purification of adult *Toxocara* worms and analysis of immuno reactive fractions using Western blotting

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**Background**: *Toxocara* is one of the most widespread roundworms worldwide listed as zoonosis pathogens. We aimed to identify the immune reactive bands of adult *Toxocara* antigens against human sera antibodies. The results might facilitate detection of human toxocariasis.

**Methods:** The crude antigens were prepared from *T. canis* and *T. cati* adult worms. Serum samples were obtained from confirmed cases of toxocariasis, and from healthy individuals and tested with SAD-PAGE and Western blotting.

**Results**: The immuno-reactivity of the naturally infected patient's sera against *T. canis* antigens showed with 16 bands from 25 to 250 kDa. The immuno-reactivity of the patients against *T. cati* antigens was observed with 7 bands 38, 40, 42, 72, 90, 100, 250 kDa. Common reactive bands were observed at 38, 40, 72, 100 and 250 kDa. Pool negative serum did not show any immune-reactivity with mentioned antigens.

**Conclusion**: *Toxocara* crude antigens showed high immunogenicity in our study on human sera. We propose that somatic antigens of *T. canis* and *T. cati* could be used as a marker in the design of diagnostic kits to determine the *Toxocara* species in positive samples after purification.

Keywords: Toxocariasis, Toxocara canis, Toxocara cati

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Radiological findings of *Lophomonas* infection in the respiratory tract: first registry-based analysis

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**Background**: *Lophomonas blattarum* is an emerging protozoan that mostly infects the lower respiratory tract and causes pulmonary lophomoniasis. Radiologic findings in patients with pulmonary lophomoniasis have yet to be studied. Thus, we conducted a registry-based clinical investigation to evaluate the radiologic findings of lophomoniasis.

**Methods:** In this registry-based study, 34 *Lophomonas* positive patients were enrolled. Demographic data, relevant characteristics, and radiologic findings of the patients were recorded and analyzed.

**Results**: Thirty-four (male = 18, female = 16) patients with an average age of  $52.21 \pm 20.48$  years old were examined. Radiological findings such as alveolar consolidation (26.5%), ground glass opacity (5.9%), centrilobular nodules (23.5%), tree- in- bud (38.2%), cavitation (23.5%), pleural effusion (23.5%), interstitial opacity (8.8%), lymphadenopathy (23.5%), bronchocele (5.9%), bronchiectasis (29.4%), nodules (8.8%) and mass (11.8%) were obtained, that the frequency of all radiological findings was less than 50%.

**Conclusion**: The most common radiological findings in patients with lophomoniasis were tree-in-bud nodules, alveolar consolidation, bronchiectasis, and centrilobular nodules that were mostly seen in the right lung and its middle and lower lobes. Given that the radiologic findings of this disease are unknown, it can be considered in the differential diagnosis.

Keywords: Radiologic findings, lophomoniasis, CT scan

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Poster

Reaction of different hydatid cyst antisera with human breast cancer tissues

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**Background**: Anti-cancer effects of hydatid cyst have been shown in different publications. However, the mechanism of action has not been clarified yet. In this work reaction of hydatid cyst fluid protoscoleces and cyst wall antibodies with the human breast cancer tissues were evaluated.

**Methods:** Reaction of sections of human breast cancer with anti-sera against hydatid cyst fluid protoscolices and cyst wall were investigated using immunohistochemistry technique.

**Results**: A strong reaction between breast cancer tissue sections and anti-hydatid cyst wall antibodies was observed. However, very faint reaction was observed when anti-sera against protoscolices or hydatid cyst fluid were used.

**Conclusion**: Results of this work showed that anti-cyst wall anti-sera reacted strongly with human breast cancer tissues. So, in treatment of breast cancer, antibodies against hydatid cyst wall can be used for selective drug delivery.

**Keywords**: Hydatid cyst, breast cancer, immunohistochemistry

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5th International & 12th National Congress of Parasitology and Parasitic Diseases of Iran		
NICOPA	Alborz University of Medical, Sciences, Karaj, Iran	
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Poster	Poster	
Retrospective study on seroepidemiology of latent infections in HIV-infected patients during the last 10 years in northeast of Iran	Revealing the hidden threat: free-living amoebae in the hospital environment of East Azerbaijan province, Iran	
Bibi Razieh Hosseini Farash <sup>1</sup> © Ø, Amin Bojdi <sup>2</sup> , Mahdi Zarean <sup>1</sup> , Pouria Hajipour <sup>3</sup> , Ghodratollah Salehi Sangani <sup>1</sup>	Hamed Behniafar <sup>1</sup> © <b>@</b> , Maryam Niyyati <sup>2</sup> , Fatemeh Mahdavi <sup>2</sup> , Soghra Valizadeh <sup>3</sup> , Ali Bahadori <sup>1</sup>	
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<b>Background</b> : Chronic and latent bacterial, viral and parasitic infections have several complications in patients with human immunodeficiency virus (HIV) .The aim of this study was to evaluate the latent infections of toxoplasmosis, hepatitis B and C, syphilis, tuberculosis, cytomegalovirus, 1-HTLV in known	<b>Background</b> : The presence of free-living amoebaes (FLAs) in a hospital poses a health risk, especially for vulnerable individuals such as patients who have undergone eye surgery. The aim of this study was to investigate the presence of FLAs in the environment, equipments and instruments of two hospitals in East-Azerbaijan province, Iran.	
patients with HIV in Khorasan Razavi province. <b>Methods:</b> In this cross-sectional study, which was conducted cross-sectionally from April 2013 to March 2023, all patients with human immunodeficiency virus, HIV, were identified in Khorasan Razavi province, whose information was available in four centers. Infectious and behavioral diseases, counseling were recorded, studied. All epidemiological information of patients and the way of HIV infection were recorded. All subjects underwent serological examination and screening tests to identify latent infections.	<b>Methods:</b> In this cross-sectional study, 40 samples were collected from two general and ophthalmology hospitals in October 2023, including 28 samples from the ophthalmology hospital (6 environmental, 8 medical equipment, and 14 sterilized medical/surgical instruments) and 12 samples from the general hospital (2 environmental and 10 medical/surgical instruments). The samples were immersed in the sterile water overnight. The water was then filtered using cellulose nitrate membranes (pore size 1.6 $\mu$ m), and the middle parts of the membranes were cultured on an <i>Escherichia coli</i> enriched 1.5% non-nutrient agar plate. After one week and up to two months, the plates were examined microscopically.	
<b>Results</b> : In this study, 255 HIV-infected people with a mean age of 41.30 12. 12.44 were included in the study, of which 164 (64.3%) were male. Most patients, 43.5%, were infected with the virus through injecting drug use. Among the patients studied, the results of serological testing and positive screening for <i>Toxoplasma</i> were 44.3%, hepatitis B 7.5%, hepatitis C 45.5%, cytomegalovirus 78.4%, syphilis 1.6%, 1-HTLV 4.7% and tuberculosis 32.6%. The mean age of patients with positive test results for <i>Toxoplasma</i> , hepatitis C and tuberculosis was significantly higher than those with negative test results ( $P < 0.05$ ).	<b>Results</b> : A total of 10 (25%) samples, including 5 medical/surgical instruments, 4 environmental samples and 1 medical device, tested positive for the genera <i>Acanthamoeba</i> , <i>Hartmannella</i> and <i>Vahlkampfia</i> (4 isolates for each genus). Among the positive plates, two related medical-surgical instruments from the general hospital showed mixed contamination with the genera <i>Hartmannella</i> and <i>Vahlkampfia</i> . The isolated amoeba genera according to hospital and type of sample were as follows: Ophthalmology hospital positive samples: 4 environmental samples (2 <i>Vahlkampfia</i> , 2 <i>Acanthamoeba</i> ), 1 device ( <i>Hartmannella</i> ), and 2 surgical instruments. General hospitals with positive samples: 3 medical instruments (1 <i>Hartmannella</i> and 2 mixed contamination).	
<ul> <li>Conclusion: This study describes the baseline rate of common latent infections in HIV-infected people in Khorasan Razavi. The results show that latent CMV, hepatitis C and <i>Toxoplasma</i> infection are more common among these patients.</li> <li>Keywords: Seroepidemiology, HIV, latent infections, Iran</li> </ul>	<b>Conclusion</b> : This study shows the presence of three genera of FLAs in hospital environments. The detection of these pathogens raises concerns about possible infections and the need for strict hygiene measures in ophthalmology hospitals. Further research and preventive measures are essential to reduce the risk of nosocomial infections associated with amoebae.	
Correspondence Email(s): Hoseinifr@mums.ac.ir	<b>Keywords</b> : Free-living amoebaes, hospital, medical/surgical instruments <b>Correspondence Email(s):</b> h.behniafar@gmail.com	



Poster

Review of retracted articles in parasitology journals over the last century

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**Background**: Retracting scientific articles functions as a method for correcting errors and addressing fraud within the scientific community. When serious and unavoidable problems such as data fabrication, plagiarism, republishing, unethical reporting, or false claims are present in a scientific article, the magazine editor has the authority to retract it.

**Methods:** The information required for this study was obtained from the "Retraction Watch" database, which serves as the reference for this topic, based on 50,601 data points spanning from 1923 to 2023. The necessary information for this study was extracted and analyzed.

**Results**: The information extracted from the "Retraction Watch" database reveals that 135 articles from parasitology journals were retracted. The first retraction occurred in 2003, and the last in 2022. On average, articles were retracted 2.8 years after their original publication. Research articles constituted 82% of the retracted articles. Authors from 52 countries contributed to the retracted articles, with significant representation from India, China, France, and the United States. The reasons for article retraction, in order, were data problems and invalidity, errors in results and conclusions, and plagiarism.

**Conclusion**: Retracted articles are crucial in the scientific world, serving as both setbacks and learning opportunities. This review delves into the reasons behind retractions, which span from unintentional errors to plagiarism and deliberate misconduct. Researchers should view retractions critically, understanding their role in advancing knowledge and promoting accountability.

Keywords: Retracted articles, parasitology journals

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Road-killed animals: resources for parasitological studies and concern for wildlife

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**Background**: There is common phenomenon to find dead animals on roadways across the world. Therefore, roadkilled animals provide an opportunity to study parasites of wildlife, including those with endangered status. Also, studying enzootic helminths are important from the perspective of public health and veterinary health.

**Methods:** Between 2021 and 2022, 41 road-killed animals were collected at different points of Ilam province, west of Iran. In total, one badger (*Meles meles*), five Jackals (*Canis aureus*), eight red foxes (*Vulpes vulpes*), eight stray dogs (*Canis familiaris*), 15 stray cats (*Felis catus*), two mice, one pigeon, and one Hedgehog were collected. The removed parasites were preserved in ethanol 70% for morphological identification and molecular phylogenetic methods. Unfortunately, most of the carcasses could not be transported to the laboratory due to severe injuries.

**Results**: The helminthological study revealed the presence of two ticks (*Haemaphisalis cocasica*, *Ixodes canisoga*), one flea (*Xenopsila* sp.), and a number of female nematode in lung of badger. *Rhipicephalus sanguineus* was found in jackals, dogs and one lice species (*Columbicola columbae*) in pigeon.

**Conclusion**: *Haemaphysalis cocasica, Ixodes canisoga*, and *Xenopsila* sp., is the newest report of badger in Iran. *Haemaphysalis caucasica* has a relatively wide range including Ukraine, southern Russia (including Crimea), Azerbaijan, the Republic of Tajikistan, Kyrgyzstan, and Iran. In the present study, a total record of 41 road kill incidents is found,

Keywords: Haemaphisalis, badger, Meles meles, fauna

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Rosa damascena extracts improve the proliferation of Leishmania major promastigotes in vitro

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**Background**: Various impacts of plant extracts on *Leishmania* parasites, including *Leishmania major*, has been an interesting subject of research. Rosemary extract has been studied mostly due to the study of its anti-parasitic properties. This study examined the in vitro effects of rosa damascena extracts on the proliferation of *L. major* promastigotes.

**Methods:** The alcoholic and aqueous extracts of Rosa damascena were extracted by maceration method and made into dry powder by freeze dryer. Then, the extracts were added in final dilutions of 50, 100, 250, 500, 750, and 1000  $\mu$ g/mL to RPMI medium containing one million promastigotes per mL. The survival of promastigotes was assessed at 24, 48, and 72 hours using microscopic techniques with trypan blue staining and Neubauer slide.

**Results**: Microscopic analysis showed that the groups treated with Rosa damascena extract had a significant increase in the number of viable promastigotes compared to the control group. Both aqueous and alcoholic extracts had similar effects in increasing the proliferation of promastigotes, but the best concentrations were 250, 500, and 750  $\mu$ g/L and the best time was 72 hours. After 72 hours, the number of parasites per/ml reached 15 million, which was 15 times greater than the initial count. Rosette-form structures with the accumulation of promastigotes were abundant and parasites were highly motile.

**Conclusion**: This study showed the positive effects of *R*. *damascena* extract on the proliferation and overall survival of *L*. *major* promastigotes, which can introduce this substance as a potential additive to improve the growth of this stage of the parasite in vitro.

Keywords: Leishmania major, Rosa damascena, proliferation

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Poster

Salt-detergent technique, a new modified formalin-ether technique for the detection of intestinal parasites

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**Background**: One of the most important diagnostic methods for intestinal protozoa in stool samples is The formalin-ether concentration technique. But due to the expensive, and dangerous solution of this technique, laboratories use it less. Therefore, we decided to reduce the costs and risks of using this method by replacing new solutions.

**Methods:** In this cross sectional study 1000 stool samples from people of Tehran city were collected. All samples were examined with both formalin ether and detergent salt methods. In final, the results were analyzed with SPSS software.

**Results**: The sensitivity of the salt - detergent technique in the detection of *Blastocystis hominis*, *Giardia lamblia* cyst, *Entamoeba coli*, and *Strongyloides stercoralis* is more than the formalinether technique, whereas in the detection of the egg of helminth such as *Hymenolepis nana*, the sensitivity of formalinether is more than salt detergent.

**Conclusion**: In this research, the results indicated that the use of the salt-detergent technique led to a more accurate diagnosis of some protozoa and worms. Since salt is much safer and cheaper than formalin-ether techniques, it can be used as a suitable substitute for formalin in laboratories.

**Keywords**: Formalin-ether, salt detergent, intestinal parasite

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Sero-molecular study on the prevalence of *Toxoplasma gondii* infection in pregnant women referred to a gynecology hospital in Urmia, Iran

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**Background**: Toxoplasmosis is a frequent infection among the human population. The infection can cause devastating complications for the fetus during pregnancy. The present study aimed to determine the serological and molecular prevalence of the infection and molecular characterization of *Toxoplasma gondii* isolates among pregnant women referred to Kowsar Hospital, Urmia.

**Methods:** In a cross-sectional study, 340 blood samples were collected from pregnant women referred to Kowsar Hospital, Urmia, Iran from May to July 2022. Anti-*T. gondii* IgG and IgM seropositivity were determined by enzyme-linked immunosorbent assay. PCR was carried out by targeting the GRA6 gene of the parasite on all patients' buffy coats. The study was approved by the Ethics Committee under the Ethical Code of IR.UMSU.REC.1401.031.

**Results**: Anti-*T. gondii* IgG and IgM antibodies were positive in two (0.6%) women, and 101 (29.7%) women had anti-*T. gondii* IgG and 70.3% were seronegative. PCR was positive in two IgM-positive women, and both isolates belonged to lineage I. The risk of infection was significantly higher in women who had constant contact with cats and soil, and who were residents of rural areas.

**Conclusion**: According to the results of the present study, the prevalence of toxoplasmosis in pregnant women in Urmia is similar to its prevalence in other areas in northwestern Iran, and despite the low prevalence of acute infection, it should not be ignored.

Keywords: Toxoplasma gondii, acute infection, genotype

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Poster

Seroepidemiology (IgG, IgM) and risk factors of *Toxoplasma gondii* in Iranian COVID-19 patients, parasite-virus-host triangle

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**Background**: Theoretically, there is a possible association between emerging SARS CoV 2 infection and parasites such as *Toxoplasma gondii* (*T. gondii*). The present study aims to evaluate the seroepidemiology of *T. gondii* in COVID-19 patients and the control group as well as its correlation with risk factors.

**Methods:** Totally, 450 sera samples were taken from COVID-19 positive patients and controls from the Tehran, Karaj, and Shiraz cities. Anti-*T. gondii* IgG and IgM were evaluated using the ELISA technique. After two months, the participants were followed for recovery or non-recovery and even death. The association between seroprevalence and severity of viral infection as well as other risk factors was statistically estimated.

**Results**: IgG prevalence in patients and healthy individuals were 59.11% and 61.77%, respectively; these values were estimated at 2.22% and 0% for IgM, respectively. There was no significant association between the prevalence of IgG with COVID-19 infection, while this association was statistically significant for IgM prevalence. The Karaj had the highest prevalence, and a significant association was observed between the seroprevalence and some variables.

**Conclusion**: There is a high prevalence of latent toxoplasmosis infection despite non-significant association and essential association of active toxoplasmosis with symptomatic forms of COVID-19 disease, and due to immunosuppressive therapies for this viral inflammatory infection, screening of these immunocompromised individuals in terms of toxoplasmosis seems necessary.

Keywords: Toxoplasma gondii, COVID-19, risk factors, Iran

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Seroepidemiology of *Toxoplasma gondii* infection in patients with liver disease in southeastern Iran

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**Background**: This study aims to investigate the prevalence of *Toxoplasma gondii* infection in patients with non-alcoholic fatty liver disease (NAFLD), as well as the effects of toxoplasmosis infection on biological biomarkers such as aspirate transaminase (AST), alanine transaminase (ALT), cholesterol (Chol), and triglyceride (Tg) levels in Sistan, southeast Iran.

**Methods:** A case-control study was conducted between December 2021 and September 2022. The study included 225 patients diagnosed with NAFLD as the case group and 225 healthy blood donors as the control group. The controls were selected from the same region and were matched with the patients based on gender and age. We collected serum samples from all patients and utilized an enzyme-linked immunosorbent assay to analyze them for the existence of anti-*T. gondii* IgG antibodies. A questionnaire and medical records were utilized to gather data on the patients' demographic factors.

**Results**: The prevalence of anti-*T. gondii* IgG antibodies were 68 (30.2%) in patients with NAFLD, whereas it was 11 (4.88%) in the control group. The seroprevalence of *T. gondii* in NAFLD patients increased in correlation with age (P < 0.001). The prevalence of NAFLD was significantly greater in the seropositive group compared to the seronegative group (P < 0.001). In addition, the levels of the metabolic markers Chol and Tg were significantly higher in *T. gondii* seropositive NAFLD patients compared to *T. gondii* seronegative NAFLD patients.

**Conclusion**: The findings of this study indicate high seroprevalence of *T. gondii* in patients with NAFLD. Further studies are warranted to investigate the underlying mechanisms and potential therapeutic implications of this association.

**Keywords**: *Toxoplasma gondii*, seroprevalence, non-alcoholic fatty

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Poster

Serological diagnosis of *Toxocara canis* in mothers and their neonate referred to selected teaching hospitals, Tehran, Iran, 2020

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**Background**: Toxocariasis is a common parasitic disease between human and animals that is mostly caused by migration or the presence of *Toxocara canis* or *Toxocara cati* larvae in tissues. Humans can get the infection through contact with infected dogs or soil or by eating contaminated food containing eggs containing parasitic larvae.

**Methods:** In this cross-sectional descriptive study, 250 mothers and 250 their neonates of age referred to selected hospitals of Shahid Beheshti University of Medical Sciences were studied. After recording the data in a special questionnaire, serum and peripheral blood smear (for eosinophil count) were collected and serum samples were tested by ELISA serological test, IgG antibody against *T. canis*. The collected data were analyzed by SPSS software version 21.00 using chi-square test (P < 0.05).

**Results**: Out of 500 patients, 24 cases (4.8%) were positive for the presence of anti-*T. canis* IgG antibody titer. Serologic positive cases included 6 cases (25%) only serologically positive mother, 6 cases (25%) only serologically positive neonates and 12 cases (50%) mother and neonate were serologically positive. Statistical analysis of the data obtained using chi-square test showed that there was a significant relationship between the positive titer of anti-*T. canis* antibody with the type of hospital and nationality (P < 0.05). On the other hand, there was no significant relationship between the positive titer of anti-*T. canis* antibody and other

**Conclusion**: The present study indicates a significant frequency of *T. canis* infection in mothers and neonates, and as a result, appropriate planning should be done to prevent this infection by the relevant authorities.

Keywords: Toxocara canis, mothers, neonate, ELISA

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Serological investigation of human cystic echinococcosis and associated risk factors in the at risk population of northeast Iran within 2018-2019

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**Background**: Hydatidosis is a leading zoonotic parasitic diseases that cause economic losses and public health concerns worldwide. The current study aimed to determine the seroepidemiological status of Hydatid cyst (HC) and assess the relationship between risk factors and this disease in high-risk individuals in Gonabd city.

**Methods:** This cross-sectional study was carried out in Gonabad health centers within 2018-2019. Sampling was done using the census method. A total of 393 serum samples were collected from 259 males and 134 females at risk for HC. A questionnaire was prepared for all participants. Demographic information such as gender, age, place of residence, education level, participation of children in animal husbandry, the way of washing vegetables, and work experience in animal husbandry were recorded. The levels of IgG antibodies were measured by enzyme-linked immunosorbent assay (ELISA).

**Results**: The infection rate was 9.7% for males and 10% for females, showing no significant correlation between gender and Hydatidosis. Among the affected population, undergraduates had a prevalence rate of 10.4%, while the 20-30 age groups had a higher rate of 16.3%. Additionally, individuals with less than ten years of experience in animal husbandry had a prevalence rate of 11.7%. Those who had contact with dogs had a prevalence rate of 7.9%, whereas individuals who did not use disinfectants to wash vegetables had a higher rate of 12%.

**Conclusion**: This study highlights the significant prevalence of hydatid cysts in at-risk individuals. This indicates the need for effective preventive measures to reduce the burden of infection. The recommendations include washing vegetables with disinfectants to eliminate any potential sources of contamination. Additionally, obtaining identification certificates for livestock dogs and collecting stray dogs.

Keywords: Echinococcosis, ELISA, prevalence, Iran

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Poster

Serological investigation of toxocariasis in pregnant women in Alborz province of Iran

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**Background**: Certain behaviors such as geophagia habit, poor personal hygiene, close contact with young dogs, and ingestion of raw meat, as well as age, and socioeconomic status, affect the prevalence of toxocariasis. In this study, we aimed at investigating the frequency of *Toxocara* infection among pregnant women and the epidemiological factors.

**Methods:** Toxocariasis-specific IgG antibodies were measured using a commercial ELISA technique in 200 pregnant women between December 2021 and May 2022. A questionnaire filled by participants included options for demographic information, gestational age, number of previous pregnancies, history of abortion, drug use, commodities, history of parasitic disease, and keeping pets.

**Results**: In total, 15 (7.5%) of the participants had anti-*T. canis* antibodies. Antibodies with high titres were the most common among people with a history of abortion and were statistically significant (P = 0.029). There was no significant association between the presence of anti-*Toxocara* antibodies and other factors (P < 0.05).

**Conclusion**: Considering the significant prevalence of toxocariasis in pregnant women as well as its health risks, preventive health measures against the toxocariasis risk factors seem more necessary.

vords:	Toxocara,	toxocariasis,	pregnant,	
seroepidemiology, Alborz				
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Serological study on the prevalence of *Toxoplasma* gondii infection in patients referred to a Medical Laboratory in Urmia, Iran

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# Iran

**Background**: Toxoplasmosis is one of most frequent infections among the human population. It can cause devastating complications for the fetus during pregnancy and immunocompromised patients. This study aimed to determine the serological prevalence of *Toxoplasma gondii* infection among patients referred to Dr. Esmailzadeh Pathobiology Laboratory, Urmia, Iran

**Methods:** In a retrospective study, 408 patients with anti-*Toxoplasma* IgG and IgM tests referred to Dr. Esmailzadeh Pathobiology Laboratory, Urmia, Iran from February 2020 to February 2024 were included in the study. Female patients in the present study were pregnant women. Anti-*T. gondii* IgG and IgM concentrations were determined by LIAISON chemiluminescent immunoassay Analyzer. Data were analyzed by SPSS version 23.

**Results**: The mean age of the patients was 31.33 (1 to 58) years old. Anti-*T. gondii* IgG and IgM were positive in 80 (19.6%), and 5 (1.2%) patients, respectively. The seropositivity in males were 24% for anti-*T. gondii* IgG and 0.0% for anti-*T. gondii* IgM and in females 19.3% for anti-*T. gondii* IgG and 1.3% for anti-*T. gondii* IgM. Anti-*T. gondii* IgG concentration was significantly higher in older patients.

**Conclusion**: According to the results of the present study, the sero-prevalence of toxoplasmosis in referrals of Dr.Esmailzadeh Pathobiology Laboratory is similar to its prevalence in other areas in Iran, and despite the low prevalence of acute infection, it should not be overlooked because of devastating effects for fetus and immunocompromised patients.

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Keywords: Toxoplasma gondii, infection, IgG, IgM, toxocariasis

Poster

Seroprevalence and associated risk factors of toxocariasis among children under 12 years in Shiraz, southwest Iran

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**Background**: Human toxocariasis is caused by the larval stage of *Toxocara canis* and *T. cati*, the nematodes in the intestine of dogs and cats, respectively. This cross-sectional study was conducted to evaluate the seroprevalence and the risk factors associated with toxocariasis in children less than 12 years in Shiraz, southwest of Iran.

**Methods:** A total of 201 blood samples were collected from children under 12 years in Shiraz. A structured questionnaire, containing sociodemographic data, was completed for each participant. Sera were evaluated for anti-*Toxocara* antibodies, using the T. canis excretory-secretory antigens prepared from the second stage larvae, in an ELISA system.

**Results**: Among the 201 participants, 96 (47.8%) were girls and 105 (52.2%) were boys. The mean age was 6.35 years ( $\pm$  3.092). Anti-*Toxocara* IgG antibodies were detected in 17 (8.46%) of the 201 children under 12 years. The association between *Toxocara* infection and gender, age, contact with dogs and cats, and the level of education of the parents were not statistically significant (*P* < 0.05).

**Conclusion**: The findings of the study revealed a relatively high prevalence rate of toxocariasis in children under 12 years. It is suggested to provide more extensive training to the general public regarding the prevention, risks, and treatment of this disease.

**Keywords**: Shiraz, Iran, children, seroprevalence, toxocariasis

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Sth International	& 12th National Congress of Parasitology and Parasitic Diseases of Iran
NICOPR	Alborz University of Medical, Sciences, Karaj, Iran
	May 21 - 23, 2024
Poster	Poster
Seroprevalence and molecular investigation of toxoplasmosis among pregnant women referred to the Tabriz Valiasr Hospital	Seroprevalence of antibodies against <i>Fasciola</i> and hydatid cyst in individuals with eosinophilia: a cross-sectional study
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<b>Background</b> : Toxoplasmosis is an infectious disease caused by the intracellular parasite <i>Toxoplasma gondii</i> that affects about one third of the world's population. <i>T. gondii</i> can transmit the infection during pregnancy to the fetus through placenta and cause congenital toxoplasmosis. Threefore, pregnant women, are important risk groups.	<b>Background</b> : Eosinophilia can be indicative of parasitic infections like fascioliasis and hydatidosis. This study aimed to evaluate the seroprevalence of antibodies (IgG) against <i>Fasciola</i> and hydatid cyst in individuals with eosinophilia, as well as to investigate potential risk factors associated with these parasitic infections.
<b>Methods:</b> The study participants comprised 185 women aged 21 - 35 years. Blood samples were collected from pregnant women, and a serological test was performed to evaluate IgG and IgM antibodies against <i>T. gondii</i> . The chemiluminescence immunoassay (CLIA) method was used to detect <i>T. gondii</i> specific antibodies. Polymerase Chain Reaction (PCR) was performed using primer RE on samples in women's blood to	<b>Methods:</b> The study included 90 serum samples from individuals with eosinophilia (6% eosinophil count), tested for IgG antibodies against <i>Fasciola</i> and hydatidosis using ELISA. Demographic information, raw vegetable and processed olive consumption, travel history to north provinces of Iran, and contact with dogs were obtained through interviews and medical records.
<b>Results</b> : The levels of IgG and IgM antibodies against <i>Toxoplasma gondii</i> were determined. The CLIA showed, 17.29% (32/185) and 3.24% (6/185) patients were seropositive for anti- <i>Toxoplasma</i> IgG and IgM Ab, respectively. The highest IgG antibody were found between the ages of 32-35 and lowest IgG 22-25 years old. PCR results indicated 32 positive cases among pregnant women. There was a significant relationship between the seroprevalence of toxoplasmosis infection and age ( $P < 0.05$ ).	<b>Results</b> : Of the 90 serum samples tested, 37 (41.1%) belonged to men and 53 (58.9%) belonged to women. Two samples (2.2%) tested positive for anti-fasciola antibodies, while three samples (3.3%) tested positive for hydatidosis. Both individuals with positive anti- <i>Fasciola</i> antibodies and three individuals with positive anti-hydatidosis antibodies reported a history of consuming raw vegetables. Additionally, one individual with anti-fasciola antibodies reported consuming processed olive. None of the individuals with anti- <i>Fasciola</i> antibodies for hydatidosis antibodies had a history of traveling to north provinces of Iran, while two individuals with hydatidosis antibodies had a history of contact with dogs
<b>Conclusion</b> : The seroprevalence rate of <i>T. gondii</i> infection in pregnant women is comparatively low. The risk factors are dependent on environmental factors. Our results confirmed that <i>Toxoplasma</i> is a common parasitic infection among pregnant women in Tabriz. Thus, it is necessary to evade the risk factors before and during the pregnancy <b>Keywords</b> : <i>Toxoplasma gondii</i> , seroprevalence, pregnant women	<b>Conclusion</b> : These results emphasize the importance of helminthic infections, in the differential diagnosis of eosinophilia, particularly in individuals with relevant dietary and environmental exposures. Public health interventions and raising awareness about the risk factors for parasitic infections, may contribute to the prevention and control of these conditions in at risk populations.
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5th International & 12th National Congress of Parasitology and Parasitic Diseases of Iran Alborz University of Medical, Sciences, Karaj, Iran May 21 - 23, 2024

### Poster

Seroprevalence of canine leishmaniasis (CanL) in sheltered dogs in Bushehr city, southwest of Iran during 2022-2023

Negar Zare <sup>1</sup>, Abdollah Najm <sup>2</sup>, Mehdi Mohebali <sup>3</sup>, Mohammad Rayani <sup>4</sup>, Zabihollah Zarei <sup>3</sup>, Maryam Bemana <sup>1</sup>, Afshin Barazesh <sup>4</sup> © **D** 

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**Background**: The Mediterranean form of visceral leishmaniasis (VL) is endemic in some regions of Iran and is often seen in children under 10 years old. There is a 90% mortality in patients, if diagnosis and treatment are not done on time. Canids, as reservoirs, play an important role in the spread.

**Methods:** Bushehr province is always mentioned as one of the endemic areas for VL, so for this purpose, as the first study in the region, 112 sheltered dogs in Bushehr city were evaluated for canine leishmaniasis (CanL) using serological Direct Agglutination Test (DAT) as well as detailed clinical examinations.

**Results**: Totally, from the 70 seropositive dogs with antibody titer of 1:320 and higher, 47 (42%) had at least one of the clinical symptoms associated with VL and considered as positive for CanL.

**Conclusion**: The current seroprevalence situation of dogs in this region, is very noticeable and can be an important alarm for policy makers and health system practitioners. More comprehensive and complementary parasitological studies should be carried out on a number of reservoirs in the region for diagnosis and treatment and to accurately

**Keywords**: Visceral leishmaniasis, sheltered dogs, Bushehr,

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### Poster

Seroprevalence of *Toxocara* spp. infection among pregnant women in Jahrom city in Fars province, southern Iran

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**Background**: Despite the importance of *Toxocara* spp. for the pregnant women, this infection is considered as a neglected tropical disease by World Health Organization. *Toxocara* spp. infection can cause systemic and ocular disease of infants during pregnancy.

**Methods:** A total of 220 serum samples were investigated for the presence of IgG antibodies against *Toxocara* spp. by enzyme-linked immunosorbent assay (ELISA). Moreover, associated factors were obtained from participant's questionnaires. Data analysis for this study was performed using the SPSS software version 24.

**Results**: Serum samples from 16.36% (36/220) of pregnant women were positive for antibodies against *Toxocara* spp. Also, there were a statistically significant differences regarding the age ( $39 \le$  years old with P = 0.009), consuming raw vegetables (P = 0.001), and eating raw or undercooked meat (P = 0.04).

**Conclusion**: In conclusion, we recommend a proper health education program for pregnant women, and the importance of serological diagnosis before pregnancy. Moreover, we believed a great need for more epidemiological studies to better understanding status of *Toxocara* spp. in pregnant women.

**Keywords**: *Toxocara* spp, seroprevalence, pregnant women, Iran

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Seroprevalence of toxocariasis in epileptic children: a casecontrol study in Southwest Iran

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**Background**: Toxocariasis caused by the infective larvae of *Toxocara canis* and *Toxocara cati* manifests as visceral larva migrans, ocular larva migrans, neurotoxocariasis, and covert toxocariasis. A relationship between toxocariasis and epilepsy has been considered. This study aimed to investigate the frequency of toxocariasis among epileptic children in compared with healthy children.

**Methods:** In the present study, 80 blood samples from children with epilepsy and 81 blood samples from healthy children were collected from the health centers of Shiraz University of medical sciences. The sera samples were evaluated for the detection of anti-*Toxocara* antibodies by ELISA using the excretory-secretory (E/S) antigens prepared from the *Toxocara* canis second stage larvae. A structured questionnaire containing the socio-demographic data was completed by participants. Statistical analysis was performed using SPSS software version 25.

**Results**: Anti-*Toxocara* antibodies were detected in serum of 17 out of 80 (21.3%) epileptic children, and 8 out of 81 (9.9%) healthy children. This study indicated that there was significant difference in *Toxocara* seropositivity between the control group and children with epilepsy (P = 0.046). Based on the statistical analysis, there was no significant correlation between IgG antibodies against *Toxocara* and variables such as age (P = 0.824), gender (P = 0.324), contact with dogs and cats (P = 0.106), pica (P = 1.000) and Literacy level of parents (P = 0.664).

**Conclusion**: Our results showed the existence of a positive association between toxocariasis and epilepsy. Further studies with larger sample numbers should be performed to better investigate the relationship between toxocariasis and epilepsy.

**Keywords**: Toxocariasis, children, epilepsy, serological method

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Poster

Seroprevalence of toxocariasis in students of Shiraz University of Medical Sciences, 2019

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**Background**: Toxocariasis is one of the most common zoonotic diseases, which is caused by the larval stage of *Toxocara canis* and *T. cati*. This cross-sectional study was conducted to evaluate the seroprevalence and the risk factors associated with *Toxocara* infection in students of Shiraz University of Medical Sciences in 2019.

**Methods:** A total of 471 venous blood samples were collected from students of Shiraz University of Medical Sciences. The sera were separated and stored at -20 °C until use. A structured questionnaire was completed by participants to obtain sociodemographic data such as gender, age, residence area, eating habits, type of washing vegetables, eosinophilia and contact with dogs and cats as toxocariasis risk factors. The sera samples were evaluated for the detection of anti-*Toxocara* antibodies by ELISA using the excretory-secretory (E/S) antigens prepared from the T. canis second stage larvae. Statistical analysiswas performed using SPSS software version 18.

**Results**: Among the 471 participants, 117 (24.8%) were males and 354 (75.2%) were females with the mean age of 20.59  $\pm$  2.15 years old. Anti-*Toxocara* antibodies were detected in 35 (7.4%) out 471 cases. The association between *Toxocara* infection rate and gender, age, residence area, eating habits, type of washing vegetables, eosinophilia and contact with dogs and cats were not statistically significant (P < 0.05).

**Conclusion**: In the current study, the seroprevalence rate of toxocariasis was reported to be 7.4% among the students of Shiraz University of Medical Sciences, and there was no significant association between gender, age, eating habits, type of washing vegetables, eosinophilia, contact with dogs and cats and residential area of students.

**Keywords**: Seroprevalence, toxocariasis, students, Shiraz University

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Seroprevalence of *Toxoplasma gondii* in accidently injured drivers who referred to Razi Qaemshahr Medical Education Center in 2023: a case-control study

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**Background**: Toxoplasmosis, a prevalent infectious, has been found to have potential effects on host behavior. It may influence certain aspects of behavior. In light of the high prevalence of toxoplasmosis in Iran, this study aims to explore its impact on the occurrence of emotional behaviors specifically in drivers involved in accidents.

**Methods:** The current study was conducted as a case-control study, wherein *Toxoplasma gondii* IgG and IgM antibodies serology was examined in a random sample of patients referred to Razi Qaemshahr Hospital. The objective of this study was to assess the levels of specific antibodies against *T. gondii* in two distinct groups. The case group consisted of 91 injured drivers, taking into consideration the existing limitations. On the other hand, the control group comprised 101 healthy individuals who did not exhibit clinical symptoms associated with toxoplasmosis. The ELISA method was employed to survey antibodies.

**Results**: Among the 91 injured individuals, 53 (58%) tested positive for *Toxoplasma* IgG, while 38 (41%) tested negative for IgG. In contrast, out of the 101 healthy participants, 35 (34.6%) were IgG positive and 66 (65.3%) were IgG negative. This discrepancy was deemed statistically significant [OR (95% CI): 2.65 (1.44, 4.87), P = 0.002]. Notably, all 192 subjects involved in the study tested negative for *Toxoplasma* IgM antibody. However, a noteworthy finding from this investigation was the significant association between *T. gondii* prevalence and marital status, with a higher prevalence of toxoplasmosis detected among married individuals in both the case and control groups.

**Conclusion**: The IgG antibody titer in the case group exhibited a notable elevation compared to the control group. This suggests a potential correlation between the presence of T. *gondii* antibodies and the manifestation of emotional behaviors in drivers, subsequently leading to an increased likelihood of driving accidents.

Keywords: *Toxoplasma gondii*, accident, toxoplasmosis, antibodies Correspondence Email(s): Eissa\_soleymani@yahoo.com Poster

Seroprevalence of *Toxoplasma gondii* infection in psychological disorders and its relationship with BMI compared to healthy group

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**Background**: *Toxoplasma gondii* is an obligate intracellular protozoan that may interfere with host lipid metabolism. *Toxoplasma* selects some aspects of host lipid metabolism. Cholesterol uptake by tachyzoites occurs through host cell endocytosis and lipoprotein pathway. This parasite may also affect obesity risk through alterations in inflammatory pathways.

**Methods:** In this study, 353 people, including 177 people with schizophrenia and bipolar disorder and 176 healthy people (controls) participated, who were examined for the serum prevalence of specific anti-*Toxoplasma* antibodies using ELISA. Their height and weight were also measured in a checklist.They were analyzed using statistical tests and logistic regression. Considering the 95% confidence interval and p 0.05, it was considered statistically significant.

**Results**: In this study, the frequency of anti-*Toxoplasma gondii* antibodies in the serum of patients and controls showed that 83 (46.9%) and 34 (19.3%) respectively, which statistically the difference between the two groups was significantly (P < 0.05). Quantitative variables of the present study, based on measuring the weight and height of the patients and the control group, it was shown that there is a significant difference in the body mass index (BMI) in the patient group in the classification of overweight and obesity compared to the healthy group (P < 0.05).

**Conclusion**: According to the results of this study, the serum prevalence of anti-*Toxoplasma* antibodies in the serum of patients with psychological disorders such as schizophrenia and bipolar is higher than the control group, *Toxoplasma* seems to be an effective risk factor for psychological disorders.

Keywords: Toxoplasma gondii infection, schizophrenia, bipolar

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Poster

Seroprevalence of toxoplasmosis in infertile men

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**Background**: *Toxoplasma gondii* is one of the most dangerous parasitic infections in immunocompromised patients. It may pass through the bloodstream to various organs, such as reproductive organs of intermediate hosts, which may lead to male infertility. Therefore, this study aimed to determine the *T. gondii* infection in infertile men.

**Methods:** In this case-control study,129 men, including 67 infertile and 62 fertile men, participated, and specific anti-*T. gondii* antibodies of IgG and IgM were detected in the serum of all cases using ELISA methods. Sperm analyses, including sperm count, morphology, motility and viscosity, were also performed on the seminal fluid.

**Results**: Based on the result of IgG antibody, a significant difference was found in toxoplasmosis in two fertile and infertile groups. Its frequency was 16.1% in the fertile group and 40.3% in the infertile group (p 0.05). Also, *Toxoplasma* infection showed a relationship with sperm count and sperm morphology, so its frequency was 72.7% in the low sperm group and 24.6% in the high sperm group.

**Conclusion**: The serum prevalence of the anti-*T*. *gondii* antibodie in infertile men is higher than the control group, and it seems that *Toxoplasma* may be an effective risk factor for causing male infertility and disruption of sperm parameters.

Keywords: Toxoplasmosis, infertility, serology, men

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Seroprevalence of toxoplasmosis in organ transplant recipients in a comprehensive transplant center in south of Iran

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**Background**: Toxoplasmosis is an infectious disease posing significant health risks, including morbidity and mortality, particularly in organ transplant recipients. This study was undertaken to evaluate the prevalence of toxoplasmosis among organ transplant recipients at a leading transplant center in the southern region of Iran.

**Methods:** A total of 150 organ transplant recipients were included in the study. Blood samples were collected using sterile techniques, and the presence of anti-*Toxoplasma* antibodies was determined through a commercial ELISA kit.

**Results**: The study cohort comprised 96 males (64.0%) and 54 females (36.0%), with an average age of 46.24 ( $\pm$ 15.13) years. The majority had undergone kidney transplants (n = 79, 52.7%), followed by liver transplants (n = 69, 46%). Only one participant had received an intestine transplant (0.7%), and another had a simultaneous pancreas-kidney (SPK) transplant (0.7%). Anti-*Toxoplasma* antibodies were found in 50 of the 150 participants (33.3%). There was no significant correlation between the presence of toxoplasmosis and the participants' sex, age, or type of organ transplant.

**Conclusion**: The study reveals a relatively high seroprevalence of toxoplasmosis among organ transplant recipients in the southern part of Iran, underscoring the need for ongoing screening for this parasitic infection in this vulnerable population.

**Keywords**: Toxoplasmosis, prevalence, organ transplant recipients

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Seroprevalence study of *Strongyloides stercoralis* infection in patients with hyperlipidemia compared to control group referring to Porsina Hospital in Guilan, northern Iran

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**Background**: Strongyloidiasis is one of the neglected diseases, which is endemic in tropical and subtropical regions of the world. Previous studies have reported an association between strongyloidiasis and some diseases. The role of lipid profile has been investigated in the survival of larval stages of helminthes, but there is limited information.

**Methods:** In the summer of 1402, participants were selected from the laboratory of Porsina Hospital in Guilan province, and their lipid profiles including TG, CHOL, LDL, HDL and VLDL were measured. Based on their biochemistry test results, participants were divided into two groups of case and control. They were also matched based on sex and age groups. Anti-*S. stercoralis* Ab (IgG) was measured by indirect enzyme-linked immunosorbent assay (ELISA), using commercially available kit (Novalisa, NovaTec, Germany). Statistical analysis was performed using SPSS version 25 and Fisher's test.

**Results**: Each case and control group consisted of 105 participants. Participant's age groups varied from 13 to 80 years old. Altogether, 56.66% of the patients were female and 43.33% were male. Based on *S. stercoralis* Ab (IgG) detection, the sero-prevalence was found 4.76% in the case group compared to 0.95% in the control group. In this study, an association between TG fall and VLDL with positive of *S. stercoralis* Ab (IgG) in hyperlipidemia group (P < 0.05) was found, but other lipid profiles did not show a significant association. Moreover, a significant relationship was found between contact with dog and *S. stercoralis*.

**Conclusion**: In this study, the sero-prevalence of *S. stercoralis* in the case group was 5 times higher than the control group. A significant association between TG and VLDL fall with *S. stercoralis* Ab (IgG) was observed, but future studies with more sample sizes are suggested to investigate.

Keywords: *Strongyloides stercoralis*, hyperlipidemia, ELISA, human Correspondence Email(s): zfk579@gmail.com Poster

Serosurvey of toxoplasmosis and its associated risk factors among adult population in Kavar District, Fars province, south of Iran: a community-based seroepidemiological study

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**Background**: Since one third of the world's population is suffering from toxoplasmosis and the prevalence of this disease in Iran is not an exception, the present study aimed to evaluate the seroprevalence of toxoplasmosis among adult population in Kavar district, Fars province, southern Iran.

**Methods:** In this cross-sectional study, a total of 1413 subjects were recruited. The demographic and background information of the subjects were collected, using a questionnaire. Blood samples were collected from the participants and sera were separated. *Toxoplasma* antigen was prepared from tachyzoites isolated from the peritoneum of mice and was used in an ELISA system for detection of anti-*Toxoplasma* antibodies. Positive *Toxoplasma* cases with native antigen were verified using a commercial kit. Data were analyzed using SPSS software, employing descriptive statistics and the *Chi-square* test.

**Results**: In a study of 1413 subjects, 50.1% were male and 49.9% were female, with a mean age of 50.12 ( $\pm$ 8.28) years. The highest number of participants (41%) were in the 46-55 age group. Anti-*Toxoplasma* antibodies were detected in sera of 155 (11%) of the subjects, of which 78 (11.02%) were male and 77 (10.92%) were female. The highest prevalence of *Toxoplasma* was observed in the age group of 46-55 (11.7%). There was no significant association between sex, age, occupation and the prevalence of toxoplasmosis (P < 0.05), while a significant association was seen between contact with cat and seropositivity to toxoplasmosis (P < 0.05).

**Conclusion**: The findings of this study indicate that the prevalence of toxoplasmosis in the studied population is relatively lower than the average prevalence of this disease in Iran.

Keywords: Toxoplasma, prevalence, Kavar, Iran

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Serosurvey of visceral leishmaniasis in organ transplant recipients in the south of Iran

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**Background**: Visceral leishmaniasis (VL) poses a significant risk of activation in transplant recipients. Fars province is a known VL endemic area and a key center for organ transplantation. This study aimed to assess the Serostatus of VL among transplant recipients at a major transplantation center in Fars province.

**Methods:** The study involved 150 recipients of organ transplants, including kidney, liver, intestine, and simultaneous pancreas-kidney (SPK) transplants. Blood samples were collected from each participant, with serum extracted and stored at -20 °C for subsequent analysis. The presence of anti-*Leishmania* antibodies in these sera was determined using an ELISA method. Data were collected through questionnaires during the sampling process and analyzed using SPSS software.

**Results**: Out of the 150 recruited patients, 96 (64%) were men and 54 (36%) were women. The mean age of participants was 46.24 ( $\pm$ 15.13). Among the participants, 79 (52.66%) had undergone kidney transplantation, 69 (46%) liver transplantation, 1 (0.66%) intestinal transplantation, and 1 (0.66%) had undergone SPK (kidney and pancreas) transplantation. Anti-*Leishmania* antibodies were detected in the sera of 4 individuals (2.7%) among the study population. The overall seroprevalence of VL did not have a statistically significant association with variables such as gender, age, type of transplant, transplant rejection, or place of residence (*P* < 0.05).

**Conclusion**: The results of the current study indicate a noticeable seroprevalence of VL in transplant patients in southern Iran. Given the potential risk of reactivation of VL in transplant patients, the need for greater attention to its prevention, timely diagnosis, and treatment becomes more obvious.

Keywords: Serosurvey, visceral leishmaniasis, organ transplant

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Poster

Significant association between rainfall rate and soil, animal and human *Toxocara* infection

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**Background**: *Toxocara* spp. are zoonotic parasites affecting both animals and humans. Eggs are excreted out through dogs and develop on soil into infective ones. The infective eggs survive longer in climates with enough moisture. In this work correlation between rainfall and *Toxocara* infection rate has been investigated.

**Methods:** In the central part of Iran two areas with high and low rainfall were selected. From these areas soil, animal fecal samples, and human sera were collected and examined for diagnosis of *Toxocara* eggs using microscopic and molecular methods.

**Results**: Result: 36.66% of soil samples in high rainfall and 14.6% in low rainfall areas contaminated with *Toxocara* eggs. The infection rates of cats in high and low-rainfall areas were 22.35% and 10.76% respectively. Infection rates in dogs were 16.42% and 6.8% for high and low rainfall areas respectively. Human infection rates in two areas were 5% for high rainfall and 1.5% for low rainfall.

**Conclusion**: The rate of *Toxocara* infection in low and high rainfall areas was different and this difference was statically significant (P < 0.05).

Keywords: Toxocara, rainfall, human, dog, cat

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Simulation of climate change and its impacts on malaria in some endemic parts of Iran by artificial neural networks (ANNs)

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**Background**: Climate change can alter the spread and pattern of infectious diseases and establish new concerns for the public health. Malaria is one of the endemic infectious diseases of Iran, whose transmission is strongly influenced by climatic conditions.

**Methods:** The effect of climate change on malaria disease was simulated by using artificial neural networks (ANNs) in Khash city, Sistan and Baluchestan province, from 2021 to 2050. General circulation models (GCMs) were employed to develop future climate scenarios under two distinct pathways (RCP2.6 and RCP8.5) in the study area. Moreover, to determine the best delay time, simulate the impacts of climate change on malaria infection and find the most important climatic parameters, ANNs were applied using daily collected data for 12 years (from 2003 to 2014).

**Results**: The future climate of the study area will be hotter by 2050. The simulation of malaria cases elucidated that there is an intense increasing trend in malaria cases under the RCP8.5 scenario until 2050, with the highest number of infections occurring in the warmer months. Rainfall and maximum temperature were identified as the most influential input variables. Optimum temperatures and increased rainfall provide a suitable environment for the transmission of parasites and cause an intense increase in the number of infection cases with a delay of approximately 90 days.

**Conclusion:** ANNs were introduced as a practical tool for simulating the impact of climate change on the prevalence, geographic distribution, and biological activity of malaria and for estimating the future trend of the disease in order to adopt protective measures in endemic areas.

Keywords: ANNs, malaria, climate change, GCMs

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Poster

Sinus infection by Lophomonas blattarum: a case report

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**Background**: *Lophomonas* blattarum, as an emerging parasite, can cause bronchopulmonary infection in humans and may result in severe respiratory symptoms. This has been rarely detected in sinuses. The correct diagnosis of this parasite and characterization of the infection are of great importance for the treatment and control of the infection.

**Methods:** In this article, a case of chronic sinusitis caused by *Lofomonas* is presented. The patient was a 33-year-old man, hospital laboratory staff, with a history of cold and sinusitis symptoms. As one of the suspected infectious agents causing these clinical conditions, *L. blattrum* was subject of investigation. In this regard, samples were taken from nasal discharge; smears were prepared and microscopic examination was performed following gram staining of the smears.

**Results**: In the direct smear microscopy, a number of live and motile *L. blattarum* with irregular and long flagella were observed and the anterior flagella bundles were clearly seen in the gram staining smear. This infection was observed twice in the patient's nasal secretions with four-month intervals, coinciding with the occurrence of a cold. However, the patient avoided administration of the drug, and this may have caused *L. blattarum* to become chronic in his sinuses and appear on occasions of nosocomial viral infection. He was advised to see a doctor as soon as he encountered nosocomial symptoms to receive effective treatment.

**Conclusion**: This is the first case of *L. blattarum* sinus infection reported from Zanjan. This infection should be considered as an opportunistic parasite in the respiratory tract and sinuses and a possible aggravating factor of other infections and is recommended to be investigated especially in cases of immunodeficiency.

Keywords: Lophomonas blattarum, sinusitis, case report

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Sth International & 12th National Congress of Parasitology and Parasitic Diseases of Iran Alborz University of Medical, Sciences, Karaj, Iran May 21 - 23, 2024

Poster

Situation and outcome of multiple parasitic infections in Iran

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**Background**: Multiple parasitic infections frequently occur all over the world particularly in the developing countries where environmental and socioeconomic parameters predispose communities to various parasitic agents. This phenomenon complicates the diagnosis, treatment, and control of parasitic diseases in endemic communities, causing significant adverse clinical and epidemiological outcomes.

**Methods:** This study aimed to explore different aspects of multiparasitism of human and tried to present a conceptual outline and a comprehensive outlook on the outcomes of multi-parasitism and missed infections in Iran, where this issue has been under considered. Concomitant parasitic infections present several challenges including misdiagnosis and underdiagnosis of human parasitic diseases and are major pitfalls in clinical parasitology labs in Iran. Reliable diagnosis is a key to the proper and effective treatment of parasitic infections, and this could be more complicated in the case of multiple parasitic infections.

**Results**: Poor diagnostic accuracy could be potentially life threatening particularly in immunocompromised people. Moreover, transmission dynamics, distribution pattern and control programs of parasitic diseases are significantly affected by underdiagnosed parasitic infections. Polyparasitism is a common feature of the epidemiology of parasitic infections in Iran, where environmental and sanitary conditions and socioeconomic parameters predispose communities to the infections. Complementary diagnostic methods could detect low-intensity infections that would have otherwise gone undiagnosed in a conventional clinical laboratory. In most laboratories in the endemic countries use of these methods is neither feasible nor cost effective.

**Conclusion**: Successful implementation of national reference labs for the diagnosis of parasitic infections is essential for the reliable diagnosis, effective treatment, and relieving burden of diseases.

**Keywords**: Underdiagnosed parasitic infections, Missed infections, mistreatment, multiple parasitic infection **Correspondence Email(s):** md.ebrahimi31@gmail.com

Poster

Soluble total antigen derived from *Toxoplasma gondii* tachyzoites increased the expression levels of NLRP1, NLRP3, NLRC4, AIM2, and the release of mature form of IL1β, but downregulated the expression of IL1β and IL18 genes in THP-1cell line

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**Background**: *Toxoplasma gondii* is an intracellular parasitic protozoan infecting homoeothermic animals and about a third of the world's population. Inflammasomes are intracellular multi-protein complexes which are activated by many factors. Inflammasomes are activated during toxoplasmosis; however, there are a lot of obscure aspects.

**Methods:** THP-1 monocyte cells were converted to M0 macrophages by PMA and treated by 100 µg/mL soluble total Ag (STAg) derived from *T. gondii* strain RH for 3 h and 24 h. After total RNA extraction and cDNA synthesis, the expression pattern of NLRP1, NLRP3, NLRC4, AIM2, IL1 $\beta$ , and IL18 was evaluated by relative realtime PCR. In addition, the cytokine release of IL1 $\beta$  and TNF $\alpha$  was evaluated in the supernatant of each well.

**Results**: The results showed statistically significant timedependent overexpression of inflammasomes. NLRP1 and NLRP3 showed the higher and lower expression, respectively, during 3 h and 24 h after exposure. Both IL1 $\beta$  and IL18 were downregulated 3 h after exposure. IL18 presented statistically significant upregulation after 24 h, but IL1 $\beta$  showed statistically significant downregulation after 24 h. The release of IL1 $\beta$  increased after 3 h, but it slightly decreased during 24 h after exposure. The concentration of TNF $\alpha$  showed an insignificant decrease compared to control, while it increased during 24 h after exposure.

**Conclusion**: Taken together, this study suggested that *T. gondii* STAg induces NLRP1 more than NLRP3, NLRC4, and AIM2. Our findings also proposed that *T. gondii* STAg downregulates the gene expression of IL1 $\beta$ , but increases the release of this cytokine. It seems that *Toxoplasma* STAg probably increase the release of IL1 $\beta$ .

**Keywords**: *Toxoplasma gondii*, soluble total antigen **Correspondence Email(s):** hosseinpazoki11@gmail.com



Some aspects of immunity against Sarcoptes scabiei

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**Background**: Scabies is a parasitic disease caused by *Sarcoptes scabiei* that burrows in the epidermis of the skin of humans and many other mammals and is considered one of the top 50 epidemic diseases and one the most common human skin diseases.

**Methods:** Erythematous papules, intense generalized pruritus, and an allergic type skin reaction, that shows features of both type I (immediate) and type IV (delayed) hypersensitivity reactions, are the main clinical signs of scabies. soluble substances (including saliva, molting enzymes and hormones, feces, and nitrogenous excretory materials) from the mites induce responses from keratinocytes, fibroblasts, macrophages, mast cells, lymphocytes, langerhans cells and other dendritic cells, and endothelial cells of the microvasculature. IgM is considered the first line of the humoral immune response. Studies show an increase in the level of IgA, IgG, and IgM in serums of the infected animals with scabies.

**Results**: In conclusion, *S. scabiei* can modulate various aspects of the mammalian innate and adaptive immune responses and it has a high impact on the health and social life of people around the globe especially in developed countries. As a result, thinking about ways to control the infection of these mites must be considered.

**Conclusion**: A comprehensive understanding of the immune events in the skin and peripheral blood occurring during scabies may provide great help for the development of diagnostic tests for scabies and a vaccine to protect against *S. scabies*, which plays an important role in the control of this parasitic disease.

Keywords: Sarcoptes scabiei, immunity, mite

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Poster

Status of major zoonotic parasitic infections in Iran and the importance of public health education in prevention of zoonotic diseases

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**Background**: The World Health Organization (WHO) has classified a group of diseases as Neglected Tropical Diseases (NTDs). NTDs have serious consequences. The aim of the present study was to review the current status of NTDs in Iran and discusses the importance of public health education in preventing them.

**Methods:** This investigation employed a systematic review approach to investigate zoonotic parasitic diseases. A comprehensive search spanning 2010-2024 was conducted across prominent academic databases (Web of Science, PubMed, Google Scholar, Elsevier, Scopus) focusing on relevant keywords "parasitic diseases," "zoonotic" and "health education". Included articles aligned with the research focus on shared infectious agents and parasitic diseases between humans and animals, while adhering to stringent quality standards encompassing study design, methodology, and result presentation. Non-English language publications, research solely focused on human-specific or nonzoonotic animal parasites, and articles outside the defined timeframe were excluded.

**Results**: Among the estimated 1500 infectious agents affecting humans, 353 are parasitic agents. The Center for Emerging Infectious Diseases highlights the role of protozoan parasites (10.7%) and helminths (3.3%) over other pathogens. In Iran, more than 85% of parasitic diseases in humans are zoonosis. Echinococcosis/hydatidosis, toxocariasis, filariasis, fascioliasis and *Leishmania*sis are the most zoonotic parasitic infections in the country. The incidence rate of hydatid cyst is from 0.6 to 1.45, and serological prevalence surveys have shown infection ranges from 2.1 to 13.7%. The prevalence of human toxocariasis infection is from 4.1 to 34.5%.

**Conclusion**: Many factors play a role in the emergence and spread of parasitic diseases, which include economic, environmental and behavioral aspects. Prevention of zoonotic parasitic diseases is much more cost-effective than treatment; the goal can be achieved with a comprehensive approach that includes public health education.

Keywords: Parasite, zoonotic diseases, health education

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th International & 12th National Congress of Parasitology and Parasitic Diseases of Iran Alborz University of Medical, Sciences, Karaj, Iran

Poster

Stereological analysis of liver and spleen of hamsters with experimental infection of live attenuated *Leishmania major* 

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**Background**: Leishmaniasis continues to be a significant health issue in many countries worldwide. The histopathological characteristics of the disease and the repercussions of the parasite on diverse tissues are still not completely understood. The main objective of this study was to assess the stereological aspects of the liver and spleen.

**Methods:** In this experimental study, attenuated *L. major* (MRHO/IR/75/ER) was prepared by continuous sub-culturing (21 times) of the parasite. Then, 24 golden hamsters were assigned into 4 groups (G1 to G4). Group 1 was the control groups, group 2 were injected with  $1 \times 108$  promastigotes of attenuated *L. major* intradermally, group 2 were received intravenously and group 4 were inoculated intraperitoneally. Four months later, the hamsters were euthanized and impression smears were prepared from the liver and spleen. The orientated method was used to obtain isotropic uniform random (IUR) sections. The tissue samples were examined by stereological methods.

**Results**: The number of hepatocyte and their nuclei volumes were significantly decreased in the *Leishmania*-infected group, compared to the control group. The number of Kupffer cells and their volume in the liver of the *Leishmania*-infected group was higher than that of the control group, and the differences were statistically significant. The volume of trabeculae and central arteries in the spleen of the *Leishmania*-infected group was lower than that of the control group and the number of lymphocytes and macrophages in the spleen of the *Leishmania*-infected group was increased compared to the control group.

**Conclusion:** Leishmaniasis leads to changes in tissue structure and their function in the host by the involvement of various organs of the immune system including the liver and spleen.

Keywords: Leishmania infantum, stereology, histopathology

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Poster

May 21 - 23, 2024

Strengthen immune responses by vaccine candidate against *Toxoplasma gondii* infection including MIC13, GRA1, and SAG1 antigens in BALB/c mice

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**Background**: Toxoplasmosis is one of the important diseases caused by the *Toxoplasma gondii* and causes serious clinical consequences. Currently, the most effective medical intervention for this disease is the use of drugs that are expensive and have side effects. So far, no effective vaccine has been provided for this parasite.

**Methods:** This study aimed to design a vaccine candidate containing B-cell and T-cell epitopes and evaluate its immunogenicity. At first, using bioinformatic tools, the immunodominant epitopes of MIC13, GRA1, and SAG1 antigens were identified, and MGS protein was designed. After cloning in a plasmid pET-28a (+), E. coli BL21 produced MGS protein as a multi-epitope chimeric antigen. This protein was used alone or with Freund adjuvant, calcium phosphate (CaPNs), and chitosan (CNs) nano-adjuvants (to enhance immunogenicity) as a vaccine in BALB/c mice. Immunogenicity of MGS, MGS-Freund, MGS-CNs, and MGS-CaPNs in case groups was compared with control groups of phosphate-buffered saline (PBS), Freund, CNs, and CaPNs.

**Results**: The results of this study demonstrated that MGS protein, alone or with adjuvants, significantly increased the dose of specific antibodies (IgG Total), especially the IgG2a subunit, the cytokine IFN- $\gamma$ , the proliferation rate of spleen lymphocytes, and the survival rate in BALB/c mice (in the challenge with the RH strain of *T. gondii*). The highest amount of antibody against the injected antigen was observed in the MGS-Freund and MGS-CNs groups and the highest concentration of IFN- $\gamma$  was seen in the MGS-Freund group. Overall, according to our results, MGS protein significantly increased Th1 and Th2 responses compared to the control groups.

**Conclusion**: According to the results of this study, MGS antigen can cause immunity against *Toxoplasma* in the murine model. Therefore, this antigen is proposed as a potential vaccine candidate, and in the future, more extensive research can be done on it along with the immunodominant epitope of other parasite genes.

Keywords: *Toxoplasma gondii*, vaccine, multi-epitope, insilico Correspondence Email(s): shahabesarvi@yahoo.com



Strongyloides stercoralis: a neglected parasite, but a serious threat to immunocompromised patients' life

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**Background**: Strongyloidiasis in immunocompromised patients can progress like a ticking time bomb to severe forms of of hyperinfection or disseminated and is potentially fatal. This study was conducted with the aim of determining the prevalence of *S. stercoralis* in Khuzestan patients and also evaluating the sensitivity of its diagnostic tests.

**Methods:** This cross-sectional study was performed from 2019 to 2020 among 300 high-risk patients in Khuzestan province, southwestern Iran. Patients with autoimmune diseases, uncontrolled diabetes, HIV/AIDS, cancer, organ transplant, hematological malignancy, asthma and chronic obstructive pulmonary disease (COPD) were examined using direct smear examination, formalin-ether concentration, Baermann funnel technique, agar plate culture, and ELISA test. Since agar plate culture was considered the reference diagnostic test, culture-positive samples were confirmed by PCR amplification and the sequencing of the nuclear 18S rDNA (SSU) hypervariable region (HVRIV) of the parasite.

**Results**: The prevalence of *S. stercoralis* infection was 1%, 1.3%, 2%, 2.7%, and 8.7% using direct smear examination, formalin-ether concentration, Baermann funnel technique, agar plate culture, and ELISA test, respectively. All culture-positive samples were confirmed by SSU-PCR. The most sensitive test was ELISA, with 100% sensitivity, followed by the Baermann funnel technique with the sensitivity of 75%. Direct smear examination, formalin-ether concentration technique, and Baermann funnel technique had the highest PPV (100%) while the ELISA had the highest NPV (100%). Significant eosinophilia was observed in the culture positive patients, who had a history of asthma, COPD, and were 60 years.

**Conclusion:** Given that the ELISA test had the highest NPV, the screening of all high-risk patients for *S. stercoralis* infection in endemic areas is recommended prior to starting corticosteroid therapy with the ELISA test. The results indicate the importance of paying attention to patients with unknown eosinophilia in endemic areas.

Keywords: Strongyloidiasis, high-risk patients, corticosteroid, Iran Correspondence Email(s): ashiri\_alireza@yahoo.com/ashiri.alireza57@gmail.com Poster

Study of *Blastocystis* prevalence in children with cancer undergoing chemotherapy

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**Background**: Immunosuppressed individuals, such as patients treated with chemotherapy drugs or patients with malignancies, are at risk for having clinical symptoms of Blastocystosis, but in healthy individuals, the disease is often self-limiting. This study aimed to evaluate the prevalence of *Blastocystis* in children with malignancies.

**Methods:** In this descriptive cross-sectional study, 52 stool specimens were collected from cancer patients admitted to the oncology ward of the Shahid Baqaei 2 Hospital, Ahvaz, Iran, for six months. To determine the risk factors and outcomes of *Blastocystis* infection, questionnaire included: demographic information, kind of cancer, gastrointestinal symptoms (such as stomach ache, cramps, nausea, vomit, and diarrhea), the appearance of stool (its color and consistency) and stool test results were designed and completed All of the patients answered the standard questionnaire. Each sample was prepared with direct smear, Lugol's iodine staining, and the formalin-ether condensation method.

**Results**: In this study, 52 stool specimens obtained from children with cancer. 53.8% of patients were male, and 46.2% were female. The frequency rate of *Blastocystis* was 21.1%. The age range of patients was between 4 months-16 years. The results showed that there was no statistically significant difference between sex, age, and *Blastocystis* infection. The results also pointed that among 52 children with cancer, 11 were infected with the *Blastocystis* parasite, and among them, six (11.5%) had gastrointestinal symptoms, including stomachache, cramps, nausea, vomit, and diarrhea. Hence, statistical analysis revealed a significant relationship between *Blastocystis* infection and gastrointestinal symptoms.

**Conclusion**: According to the detection of a high prevalence of *Blastocystis* in chemotherapy drug receivers, it can be suggested that patients with cancer undergoing chemotherapy should be screened for opportunistic pathogens such as Blastocystis. Hence, it not only prevents the person-to-person transmission but also will help increase patients' response to treatment.

Keywords: Blastocystis, children, cancer, immunocompromised

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Study of prevalence intestinal parasitic infections in Boukan city with an emphasis on rural areas, west Azarbaijan province

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Background: Intestinal parasites are one of the major causes of infection in underdeveloped areas especially in rural regions with inadequate hygiene levels.

Methods: In the present cross-sectional study, 2051 people of Bukan city were selected randomly, and stool samples were studied by the direct microscopic method, formalin-ethyl acetate on nutrient agar culture (to check Strongyloides stercoralis). Demographic characteristics of people such as: age, gender, educational status, place of residence, drinking water status were recorded in the information form. After recording the data, the results were statistically analyzed.

Results: In this study, 28 (4%) of 621 samples from rural residents and 24 (1.7%) samples from 1430 urban residents were infected with intestinal parasites. Out of these cases, 14 cases were infected with Entamoeba histolytica trophozoites, 3 cases of Entamoeba histolytica cysts, 4 cases of Entamoeba coli cysts, 6 cases of Giardia lamblia cysts, and 5 cases of Giardia lamblia trophozoites, 7 cases of Blastocystis hominis, 2 cases of Taenia saginata, 1 case of Diantamoeba fragilis. 1 case of Hymenolpis nana, 1 case of Trichomonas hominis, 3 cases of Cryptosporidium, 4 cases of Endolymax nana.

**Conclusion**: It seems that the prevalence of intestinal parasites has decreased a lot compared to the past, which is due to the observance of health issues in the society. Providing health care in the study area has been effective in these results.

Keywords: Intestinal parasites, infection, Bukan, formalinether

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Background: Medicinal plants have been used in malaria treatment for many years, and they may modulate the immune system besides antiplasmodial effects to facilitate parasite clearance. This study investigated the in vivo antiplasmodial and immunomodulatory activities of Dracocephalum kotschyi essential oil.

Methods: The essential oil was extracted using a Clevenger, and the chemical content of this oil was determined using GC-MS. Five groups of six Balb/c mice were infected with Plasmodium berghei and treated with 50, 100, and 150 mg/kg of the essential oil intraperitoneally based on 5-day, and prophylaxis protocols, and 25 mg/kg Chloroquine and 0.1% DMSO were positive and negative controls. The serum levels of pro-inflammatory and antiinflammatory cytokines were measured using ELISA in the prophylaxis test. Furthermore, histopathological sections from mice's livers, spleens, and kidneys were examined to follow the changes.

Results: Z-citral and methyl geranate were the two main constituents of this oil. In the 5-day test, the mean percentage of parasite suppression was 73.31%, 80.99%, and 85.12% at 50, 100, and 150 mg/kg, respectively. Furthermore, in the 5-day test, the 150 mg/kg of essential oil showed 77.05% parasite suppression on day 7 after infection. Additionally, the percentage of immunomodulatory activity of 150 mg/kg D. kotschyi EO for IL-6, IL-10, TNF-α, and IFN-γ was 47.27%, 46.21%, 30.45%, and 68.3%, respectively.

**Conclusion**: According to this study, it can be concluded that the EO of D. Kotschvi not only prevents the growth and multiplication of the parasites but may also be effective in defending against parasites by boosting the immune system of the host.

Keywords: Plasmodium, Dracocephalum kotschvi, Essential oil Correspondence Email(s): hejazi@med.mui.ac.ir

Poster

Study of the antiplasmodial effect of Dracocephalum kotschyi essential oil on mice infected with Plasmodium berghei

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5th International & 12th National Congress of Parasitology and Parasitic Diseases of Iran Alborz University of Medical, Sciences, Karaj, Iran

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#### Poster

Study of the effects of the recombinant *Toxoplasma gondii* profilin in induction of pro-inflammatory response by macrophages via NLRP3-mediated signaling pathway; in vitro model

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**Background**: One of the most important proteins of *Toxoplasma gondii* for movement and invasion is profile. Considering the role of inflammasomes, especially NLRP3 (NACHT, LRR, and PYD domains-containing protein3) in the immune response to *T. gondii* and autoimmune diseases, we hypothesized that the *T. gondii* profile could play a role in the development producing IL1 $\beta$ , IL18, TNF $\alpha$ , and inflammation.

**Methods:** THP-1 cells were cultured in RPMI 1640 complete medium, then sorted into five groups, and treated, including Group 1, which used MCC950 (with blocking role). In group 2, MCC950 with recombinant profilin was used. Group 3 which THP-1 cells were treated only with profilin: positive control group (treated with LPS) and negative control group (without treatment). Real-time PCR and ELISA investigated NLRP1, NLRP3, NLRC4, Caspase1, IL1 $\beta$ , IL18, and TNF $\alpha$ . To confirm inhibition of the NLRP3 pathway by MCC950 and Real-time PCR and ELISA results, a Western blot test was performed with NLRP3 and IL1 $\beta$  Abs.

**Results**: Real-time PCR showed that profilin significantly increased the expression of the IL1 $\beta$  gene 5 hours after treatment. ELISA results also showed that this protein significantly increases the secretion of cytokines TNF $\alpha$  and IL1 $\beta$  by THP-1 cells. Western blot's results of IL1 $\beta$  protein production by this test were consistent with the results of Real-time PCR and ELISA. The results of IL1 $\beta$  confirm the effect of NLRP3 as the main inflammasome for inflammasome with MCC950, a significant decrease in IL1 $\beta$  expression was observed by all three tests.

**Conclusion**: Our results show that the NLRP3 inflammasome plays an important role in producing IL1 $\beta$ , IL1 $\beta$ , TNF $\alpha$ , and inflammation by *T. gondii* recombinant protein. According to the information of this study, it can be concluded that *T. gondii* recombinant protein can play a special role in activating the human immune system.

Keywords: *Toxoplasma gondii*, profilin protein, cytokine, Correspondence Email(s): hosseinpazoki11@gmail.com Poster

Study of the frequency of blood protozoa in sheepdogs and watchdogs in some rural areas of Shirvan city

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**Background**: Canine babesiosis and hepatozoonosis are essential worldwide, tickborn diseases caused by apicomplexan haemoprasitic protozoa from genera *Babesia* and *Hepatozoon*. This study aimed to evaluate the prevalence of *Hepatozoon* spp. and *Babesia* spp. infection in some rural areas of Shirvan city, northeast of Iran.

**Methods:** 264 blood samples were taken from apparently healthy sheepdogs and watchdogs in the spring of 2021. Thin blood smears were prepared, stained by Giemsa, and examined with a light microscope. Gender, age, breed, origin, and tick infestation status were recorded in a questionnaire. The parasite level of dogs infected with *Hepatozoon* was determined by counting 200 neutrophils in the microscopic field. Also, the level of parasitemia of Babesia was determined by observing 50 microscopic fields. The association between the prevalence of haemoprotozoa and host factors was analyzed by *Chi-squared* test, and *P*-values of 0.05 were considered statistically significant.

**Results**: The haemoprotozoa identified were small *Babesia* species and *Hepatozoon canis*. The overall prevalence of blood protozoa infection in the studied dog population was determined in 22 cases out of 264 samples (8.3%); 2.7% (7/264) of dogs were infected with small *Babeisa* species, and 5.3% (14/264) were infected with *H. canis*. One dog (0.38%) had a simultaneous infection with small *Babesia* species and *H.canis*. The level of parasitemia of Babesia was determined in the range of 0.005-0.02 %, and the parasitemia level of *Hepatozoon* ranged between 0.5-3.5 %. 16.6% of dogs (44/264) were infested with ticks.

**Conclusion**: Based on statistical analysis, the frequency of *Hepatozoon* spp. or *Babesia* spp. infection had no significant difference with the factors of gender, age, and tick infection (P < 0.05). The results of this study indicated that the prevalence and parasitemia of blood protozoa in dogs were low.

**Keywords**: Babesiosis, hepatozoonosis, *Hepatozoon canis*, sheepdogs

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Study of therapeutic effect of combined topical fluconazole plus glucantime compared to glucantime injection alone in the treatment of acute cutaneous leishmaniasis

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**Background**: Cutaneous leishmaniasis (CL) is an endemic disease in Iran with no available vaccine. Due to the similarity between the membrane of fungi and the cell wall of *Leishmania* and the same function of fluconazole in causing a disorder in them, this study aimed to assess fluconazole's efficacy in CL treatment.

**Methods:** In a double-blind prospective clinical trial, 100 CL patients were studied in two groups. Group A was treated with topical fluconazole twice daily along with intralesional glucantime once a week and group B, topical placebo twice daily with intralesional glucantime once a week for a maximum of 6 weeks. Patients were followed for 3 months. Data were statistically analyzed using the chi-square test.

**Results**: In the group treated with fluconazole along with glucontime, 32 cases (64%) had complete recovery, while in the group treated with glucontime and topical placebo, 19 patients (38%) achieved a complete cure. According to the chi-square statistical test, this difference was significant (P < 0.01).

**Conclusion**: Combining fluconazole with glucantime showed superior efficacy compared to placebo plus glucantime in CL treatment. Fluconazole also decreased healing time and required glucantime dosage. According to the results of the study, it can be acknowledged that using fluconazole in the treatment of CL reduces the side effects of glucantime.

Keywords: Cutaneous leishmaniasis, fluconazole, glucantime

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Poster

Study on epidemiological situation and annual incidence rate of cutaneous leishmaniasis in Shushtar city, Khuzestan province between years 2009-2023

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**Background:** Cutaneous leishmaniasis caused by protozoan *Leishmania*, including two agents, *Leishmania major* (zoonosis) and *Leishmania tropica* (anteropontic) which there is extensively in the world also in the most cities of Iran. This study aimed to investigate on epidemiological situation and annual incidence rate of cutaneous leishmaniasis (CL) in Shushtar Health Center.

**Methods:** This retrospective and cross-sectional study is based on the recorded data of suspected to CL that referred to Health Center which were collected and analyzed. In this center the smears were examined by Giemsa staining and under microscope with  $100 \times$  magnification.

**Results:** Out of 1133 cases of total samples, 490 cases (43.20%) of which 286 cases (58.36%) were male and 204 cases (41.63%) female have been diagnosed positive. The most positive cases of CL were observed in 2011 (75%) and 2012 (65.96%), and the least positive cases were confirmed in 2023 (23.07%).

**Conclusion:** Men are more exposed to contamination compared to women due to their job and the way they cover their bodies. Considering the relatively high statistics of contamination and the endemicity of the region with two important conditions, the reservoir of rodents and sand flies, can be significant in the occurrence.

**Keywords:** Epidemiology, cutaneous leishmaniasis, Shushtar

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Study on the seroprevalence of cystic echinococcosis in rural population referring to Reference Laboratory in Urmia

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**Background:** Cystic echinococcosis is considered one of the important zoonotic parasitic diseases worldwide. The present study aimed to determine the seroprevalence of cystic echinococcosis in the rural population referred to Reference Laboratory in Urmia, northwest Iran.

**Methods:** In this cross-sectional study, 698 sera were collected from the rural population referred to the Reference Laboratory in Urmia from January to May 2023. Sera were tested for anti-*Echinococcos* IgG using an enzyme-linked immunosorbent assay (ELISA) kit.

**Results**: Eight humans (1.1%) were positive for anti-*Echinococcos* IgG. The frequency of cystic echinococcosis in males and females was 6(3.1%) and 2(0.8%), respectively.

**Conclusion**: Because of the low positive rate, none of the demographic variables were statistically significant as a risk factor.

**Keywords**: Cystic echinococcosis, ELISA, IgG, Iran

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Study on the seroprevalence rate of toxocariasis in rural population referring to Reference Laboratory in Urmia.

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**Background**: Toxocariasis is a prevalent zoonosis with a significant socioeconomic impact, particularly on impoverished communities around the world. The present study aimed to determine the seroprevalence of toxocariasis in the rural population referred to Reference Laboratory in Urmia.

**Methods:** In this cross-sectional study, 348 sera were collected from the rural population referred to the Reference Laboratory in Urmia from January to May 2023. Sera were tested for anti-*Toxocara* IgG using an enzyme-linked immunosorbent assay (ELISA) kit.

**Results**: Eighty-seven individuals (19.9%) were tested seropositive for toxocariasis. Sex (P = 0.036), method of washing vegetables (P = 0.037), and contact with dogs (P = 0.012) were found to be the risk factors associated with toxocariasis in the studied area.

**Conclusion**: Based on the results of the present study, toxocariasis is highly prevalent and alarming in rural areas of Urmia, North West Iran. Therefore, health education, prevention, and control programs seem to be necessary to educate the people living in the area.

Keywords: ELISA, IgG, toxocariasis, Iran

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Survey of free -living amoeba and *Acanthamoeba* in nasal samples of cancer patients, Kashan, Iran 2019-2020

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**Background**: Acanthamoeba is a Free–Living Amoeba (FLA) that are abundant in the nature. This opportunistic protozoan, can produce amoebic keratitis and granulomatous encephalitis in immunocompromised patients. Due to little information about the status of this parasite, present study was performed to determine the prevalence of FLA and Acanthamoeba in cancer patients.

**Methods:** This cross-sectional study was performed on one hundred nasal mucosal samples of cancer patients referred to Shahid Beheshti and Yathribi hospitals in Kashan during 2019 to 2020. The samples were cultured onto 1.5% Non-nutrient agar enriched with killed Escherichia coli and examined for the presence of FLA. After mass cultivation of FLA and DNA extraction, Acanthamoeba spp. were determined by PCR using JDP1 and JDP2 primers, which amplified a 500 bp fragment. After recording data in questionnaire, the prevalence of FLA and *Acanthamoeba* were determined.

**Results**: Finding of the present study showed that the prevalence of FLA in nasal mucosal samples of cancer patients was 89%. The rate of FLA in women and men were 92.2% and 85.7% respectively (P = 0.3). The highest rate of FLA contamination was observed in patients more than 66 years (95%), but the difference was not significant. In addition, the rate of FLA according to the education and job were not significant (P = 0.8, P=0.4) respectively. The prevalence of *Acanthamoeba* sp. in nasal mucosal samples was 38%.

**Conclusion**: The rate of free-living amoeba and Acanthamoeba in nasal mucosal samples of cancer patients in Kashan were more than the study results of Iran and the world. Therefore, health education about transmission and improving sanitation is recommended for prevention of infection.

Keywords: Free- living amoeba, *Acanthamoeba*, cancer Correspondence Email(s): rasti\_s@yahoo.com

Poster

Survey of parasitic contamination in vegetables raw consumed in Birjand city in 1402

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**Background**: Vegetables are a part of the diet of the people of the world, which contain essential vitamins for the body. During the stages of planting, harvesting and supply to the market, they are always at risk of contamination with different types of parasites and have a high potential for transmission.

**Methods:** In this descriptive-analytical study, 200 samples of vegetables were collected from Birjand city in 1402. 250 gr samples of each type of vegetable were taken to the laboratory. The samples were dissolved in buckets containing 1 liter of physiological serum and the sediments were centrifuged. The contents of the supernatant were slowly thrown away and examined by the laboratory method of direct smear and preparation of smear and staining with Lugol under a microscope with 10 and 40 magnification. Analysis and analysis of data using spss software and by statistical test and test Fisher's exact test was performed.

**Results**: 78 (39%) vegetables were infected with parasites. The highest frequency distribution of parasitic infection was related to spinach (70%), almonds leeks (66%), mint (53%), coriander (44%), lettuce (50%) and leeks (29%). The most frequent explanation of parasitic infection was related to *Blastocystis* (23.5%), *Giardia* (2%), nematodes (2%), *Dicrocuelium* eggs (1.5%) and *Entamoeba* spp. cyst (1%). No significant relationship was observed between the frequency distribution of parasitic infection and each of the variables of the season, distribution areas and breeding place (P > 0.05), but a significant relationship was observed with the type of vegetables consumed (P < 0.05).

**Conclusion**: According to the results and the high level of contamination of vegetables, appropriate disinfection of vegetables before consumption and training of community members in Birjand city should be done.

Keywords: Vegetables, Birjand, parasitic contamination

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Survey of the seroprevalence of toxoplasmosis among childbearing-age women in Mazandaran province and its possible correlation with vitamin D deficiency

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**Background**: Toxoplasmosis which is caused by the protozoan parasite *Toxoplasma gondii*, affects pregnancies in women. Vitamin D deficiency increases susceptibility to infections and complications during pregnancy. This study investigated the prevalence of toxoplasmosis in women of reproductive age in Mazandaran province and explored the possible correlation between vitamin D deficiency.

**Methods:** A total of 320 serum samples of childbearing-age women in Mazandaran province (Sari, Babol, Chalus, Nur, Tonekabon, and Ramsar) were collected. Participants completed a questionnaire providing information including age, meat consumption, cat exposure, egg consumption, soil contact, and residential location. The sera were tested for IgG and IgM antibodies against *T. gondii* using ELISA, and 25-hydroxyvitamin D levels were measured using a commercial kit. The results were analyzed using descriptive statistics and the *Chi-square* test, as well as calculating the odds ratio, utilizing Spss software version 21. The *P* < 0.05 was considered statistically significant for all tests.

**Results**: This study showed that 198 cases (61.88%) of the studied population had anti-*Toxoplasma* IgG, while one case (0.31%) had anti-*Toxoplasma* IgM. Among the women who had insufficient vitamin D, 159 cases had anti-*Toxoplasma* IgG and one sample had anti-*Toxoplasma* IgM. Also, in people who had sufficient vitamin D, 39 samples had anti-*Toxoplasma* IgG, and no sample had anti-*Toxoplasma* IgM. Statistical analysis showed that the prevalence of toxoplasmosis in people with insufficient vitamin D is 1.71 times higher than those with sufficient vitamin D (OR = 1.71) and there is a significant relationship between the prevalence of toxoplasmosis and vitamin D levels.

**Conclusion**: High *T. gondii* prevalence in women of Mazandaran suggests the parasite's life cycle is established there. Lower vitamin D levels increase toxoplasmosis risk, which is important for seronegative women during pregnancy, due to its dangerous effects on the fetus and mother. Therefore, monitoring vitamin D in seronegative women is crucial.

Keywords: Toxoplasmosis, Vitamin D, childbearing-age, women Correspondence Email(s): hosseini4030@gmail.com Poster

Survey on *Neospora caninum* presence in retailed small ruminants' livers in Gonbad-e-kavoos, northern Iran

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**Background**: *Neospora caninum* is an apicomplexan parasitic agent of neosporosis with globally distribution. Concerning the relationship of *N.caninum* and *T. gondii* and the seropositivity reports, the pathogenicity of the *N. caninum* in humans is discussible. This survey was conducted to potential risk evaluation of retailed small ruminants' livers in Gonbad-e-kavoos city.

**Methods:** In this cross-sectional descriptive study, the livers of 117 small ruminants, consisting of 97 sheep and 20 goats, were sampled from Gonbad-e-kavoos slaughterhouse. DNA extraction was done by validated commercial kits and using the designed primer (targeting NC5 marker genomic sequence) the desired fragment was amplified by Real Time-PCR.

**Results**: Out of 117 liver samples, 5 samples (4.3%) were infected with N.caninum, including 4 (4.1%) sheep and 1 (5%) goats. Five out of 65 (7.7%) male animals' livers but no sample of female animals were detected as PCR-positive. Also, all cases were in the age group of 9 to 24 months. No statistically significant difference was found about the prevalence of *N. caninum* infection with age, sex and type of examined ruminants (P < 0.05).

**Conclusion**: The results of this survey confirm the presence of *N. caninum* in the livers of sheep and goats (regardless of age and gender) and as the most popular edible organ in the area's inhabitants and emphasize the necessity to avoid consuming raw or undercooked livers of ruminants to prevent human infection.

Keywords: Neospora, sheep, goats, liver, Gonbad-ekavoos

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Survey on the seroprevalence of *Toxoplasma gondii* among people with vitamin D deficiency and people with normal vitamin D level in Shahroud, 2022

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**Background**: Considering the importance of toxoplasmosis in people with vitamin D deficiency and its complications, the current study was designed to investigate the prevalence of *T. gondii* among people with vitamin D deficiency and people with normal vitamin D in Shahroud in 2022.

**Methods:** The present study was a cross-sectional descriptive study. The studied population was 181 people in two groups including 91 people with vitamin D deficiency (vitamin level less than 10 ng/ml) and 90 people with normal vitamin D. Serums were evaluated to determine the level of IgG and IgM antibodies of *T. gondii* using ELISA kit. Finally, the test results and questionnaire data were statistically analyzed by SPSS 23 software.

**Results**: Findings showed that people with vitamin D deficiency had 21% positive IgG immunoglobulin, while normal people had 10% positive, and this difference was statistically significant (P = 0.034). In addition, 11% of people with vitamin D deficiency had positive IgM immunoglobulin, while normal people had 3.3% were positive and this difference was statistically significant (P = 0.046).

**Conclusion**: The results of this study showed that the serum level of *T. gondii* in people with vitamin D deficiency is significantly higher than people with normal vitamin levels in Shahroud.

Keywords: Prevalence, Toxoplasma, vitamin D, infectious

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Taxonomic identification of ectoparasites of Afshari sheep in Kashmar

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**Background**: The Afshari sheep breed is wellknown in Kashmar. Ectoparasite infestations in livestock farms in Khorasan Razavi province are a major concern. Arthropods, a diverse group of invertebrates constituting over 80% of all animal species, inhabit nearly every habitat. Parasites that can infect sheep skin include lice, mites, ticks, and fly larva.

**Methods:** Wool loss, and skin inflammation may necessitate laboratory tests for accurate diagnosis. This study focused on 150 Afshari sheep. External parasite samples were isolated using standard methods and sent to the parasitology laboratory at Ardakan University.

**Results**: All isolated parasites were identified as Ixodidae ticks, comprising 36.7% *Ripicephalus turanicus*, 31.3% *Hyalomma anatolicum*, 14.7% *Hyalomma marginatum*, and 3.3% *Ripicephalus bursa*.

**Conclusion**: Common clinical signs of infection such as burning, wool loss, and skin inflammation may necessitate laboratory tests for accurate diagnosis. The results, along with age differences, indicated a higher infestation rate in younger sheep, suggesting a significant correlation.

Keywords: Ectoparasite, Afshari sheep, Kashmar

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Sth International	& 12th National Congress of Parasitology and Parasitic Diseases of Iran
NLCOPR	Alborz University of Medical, Sciences, Karaj, Iran May 21 - 23, 2024
Poster	Poster
TGF-β targeted by miR-27a modulates anti-parasite responses of immune system	The anti- <i>Trichomonas</i> effects of nano emodin extracted from <i>Rhamnus cathartica</i> on <i>Trichomonas vaginalis</i> : an in vitro study
Faezeh Hamidi <sup>1</sup> ©, Samira Mohammadi-Yeganeh <sup>2</sup> , Mostafa Haji Molla Hoseini <sup>3</sup> , Seyyed Javad Seyyed Tabaei <sup>4</sup> , Niloofar Taghipour <sup>5</sup> , Ameneh Koochaki <sup>3</sup> , Vahedeh Hosseini <sup>6</sup> , Ali Haghighi <sup>4</sup> ©	Elham Kia Lashaki <sup>1</sup> © Ø, Aroona Chabra <sup>2</sup> , Bahman Rahimi Esboei <sup>3</sup>
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Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran; <sup>6</sup> Department of Molecular Medicine and Genetics, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran	<b>Background</b> : Trichomoniasis is one of the common diseases of the urogenital system caused by <i>Trichomonas vaginalis</i> . Due to the cytotoxic effects of medicinal plants such as <i>Rhamnus cathartica</i> on microbial organisms, this study aimed at evaluating nano emodin extracted from Rhamnus cathartica plant on the growth of <i>Trichomonas</i> in vitro.
<b>Background</b> : Immune cells and their secreted cytokines are known as the first barrier against pathogens. <i>Leishmania major</i> as an intracellular protozoan produces anti-inflammatory cytokines that lead to proliferation and survival of the parasite in the macrophages. miRNAs are small non-coding RNA molecules that regulate mRNAs expression. We aimed to investigate the relationship between the expression of TGF- $\beta$ and a bioinformatically candidate miRNA, in leishmaniasis as a model of TGF- $\beta$ overexpression.	<b>Methods:</b> The plant was approved in herbarium and hydro- alcoholic extracts were prepared. The experiment was done using 24 wells cell culture plate. In each well, TYM culture medium and different concentrations of plant extract was added. Also, metronidazole was added to positive control well. Then TYM were added to all wells containing 500,000 parasites and the plates were incubated at 37 °C. The experiment was performed as double blind
<b>Methods:</b> The miRNAs that target TGF- $\beta$ -3 'UTR were predicted and scored by bioinformatic tools. After cloning of TGF- $\beta$ -3'UTR in psi- CHECK TM- 2 vector, targeting validation was confirmed using luciferase assay. After miRNA mimic transfection, the expression of miR-27a, TGF- $\beta$ , as well as nitric oxide concentration was evaluated. <b>Besults:</b> miR-27a received the highest score for targeting TGE- $\beta$ in	and triplicate. The mean number of the parasites in different concentrations, times and effect of the extract were recorded. <b>Results</b> : Total extract of Rhamnus cathartica and Nano Emodin in all concentrations have had acceptable antiparasitic effects against <i>T. vaginalis</i> parasite. In concentrations of 400 and 800 μg/mL of nano emodin and concentration of 800 μg/m of <i>Rhamnus</i>
bioinformatic predictions. luciferase assay confirmed that mR-27a it argeting TGF- $\beta$ -3'UTR, since miR-27a transfection decreased the luciferase activity. After miRNA transfection, TGF- $\beta$ expression and nitric oxide concentration were declined in <i>L. major</i> infected macrophages.	<i>cathartica</i> plant extract, better effects than the positive control have been reported. <b>Conclusion</b> : Considering the killing power of Rhamnus cathartica whole plant extract and Nano Emodin on <i>Trichomonas</i> and its
<b>Conclusion</b> : Bioinformatic prediction, luciferase assay, and miRNA transfection results showed that miR-27a targets TGF- $\beta$ . Since miRNA and cytokine-base therapies are developing in infectious diseases, finding and validating miRNAs targeting regulatory cytokines can be a novel strategy for controlling and treating leishmaniasis.	better effect than the positive controls, it can be concluded that the above compounds can be suitable candidates for treatment after further studies. <b>Keywords</b> : <i>Trichomonas vaginalis</i> , <i>Rhamnus cathartica</i> , anti- <i>Trichomonas</i>
Keywords: Leishmania major; luciferase assay, immunity	Correspondence Email(s): kia_e65@yahoo.com
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	1



The candidate biomarkers of salmonids fish infected by parasite *Tetracapsuloides bryosalmonae*: a bioinformatics study

Seyed Mohammadmahdi Meybodi<sup>1</sup> © **P** 

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**Background**: *Tetracapsuloides bryosalmonae* is a salmonids fish endoparasite and causes proliferative kidney disease (PKD) in them. However, the molecular mechanism of *T. bryosalmonae* infection is not well understood.

**Methods:** After obtaining the gene expression omnibus (GEO) microarray profiling dataset, the GEO2R analyzer tool was employed to identify differentially expressed genes (DEGs). Furthermore, Search Tool for the retrieval of interacting genes (STRING) was used to conduct protein-protein interaction (PPI) network and gene ontology (GO) or biological process enrichment analysis. Then, candidate hub genes were obtained as biomarkers using Cytoscape software.

**Results**: Of a total of 195 recognized DEGs, 168 and 27 were upregulated and downregulated genes, respectively. GO enrichment on upregulated genes identified biological processes like cellular defense response and immune system processes. Then, the database of Kyoto Encyclopedia of Genes (KEGG) was used to recognize the enriched pathways, and pathways including pentose phosphate pathway, metabolic pathways, and apelin signaling pathway were identified. Moreover, after the extraction of genes from the PPI network of rainbow trout infected by T. bryosalmonae, the five mostly involved hub genes (dhx58, cmpk2, IFI44, irf7, and mx2) ranked by score in Cytoscape.

**Conclusion**: In conclusion, identification of these genes increases our knowledge about the molecular mechanisms caused by T. bryosalmonae infections in fishes and could be the potential targets for treatment/ early diagnosis of parasite infections. By employing bioinformatics analysis, this research serves as a hypothesis and calls for further clinical examination.

Keywords:	<b>Bioinformatics</b> ,	Tetracapsuloides
bryosalmonae,	biomarker, fish	

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Poster

The comparison of activated sludge and stabilization ponds efficiency in removal of parasite eggs and protozoan cysts

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**Background**: This study aims to determine the efficiency of the wastewater treatment plants in the north of Isfahan and Fouladshahr stabilization ponds in removing parasitic contamination (protozoan cysts and worm eggs).

**Methods:** This study was a cross-sectional description study that was conducted at a one-year period on wastewater treatment plants in North Isfahan and Fouladshahr; Iran. Sampling was done from influent and effluent of two WWTP. To check the presence of parasite eggs and protozoan cysts three methods include of direct slide, concentration with formalin ether and Sheather's was used. To check the presence of coccidian the specific methods of Trichrome staining and modified acid-fast (warm) were used. Parasite eggs and protozoan cysts were counted by the Ballenger's method and using the McMaster slide. Finally, the efficiency was analyzed using statistical tests.

**Results**: human Ascaris eggs, animal Ascaris eggs, *Trichostrongylus* species eggs, other worms' eggs and nonparasitic free-living worms that shown in Table 2. The average efficiency of four seasons of North Isfahan WWTP in removing human Ascaris eggs was 94%, animal Ascaris eggs was 95%, *Trichostrongylus* species eggs was 98%, other worm eggs was 95% and eggs, larvae and adult worms were non-parasitic was 94%. while in Fouladshahr WWTP the removing of human Ascaris eggs was 95%, animal Ascaris eggs was 97%, *Trichostrongylus* species eggs was 95%, other worms eggs was 100% and non-parasitic eggs, larvae and adult free-living worms was 99%.

**Conclusion**: The average number of nematode eggs and adult worms in free-living in the effluent of the north Isfahan WWTP has a significant difference with the effluent standard, but in Fouladshahr WWTP, there is no significant difference with the effluent standard.

Keywords: Wastewater treatment, worm egg, cyst

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The comparison of *Entamoeba gingivalis* and *Trichomonas tenax* prevalence between gingivitis patients and without gingivitis individuals: a case-control study

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**Background**: *Entamoeba gingivalis* and *Trichomonas tenax* can easily proliferate into the gingival tissues as opportunistic organisms during periodontal disease. in the present study we decided to evaluate the association of *E. gingivalis* and *T. tenax* prevalence between gingivitis patients and without gingivitis individuals.

**Methods:** In this case-control study, 350 patients (175 with gingivitis and 175 without gingivitis) over 14 years referred to the Sari Dental School Clinic were selected. The samples were stained under two methods of giemsa and trichrome staining. After staining and rinsing, the slides were dried and examined under a microscope at  $100 \times$  magnification for at least 15 minutes. *T. tenax* or *E. gingivalis* was reported. Qualitative variables were compared between case and control groups with *Chi-square* and Fisher's exact tests. Univariate and multivariate logistic regression analysis was also performed.

**Results**: The results showed that the prevalence of all parasitic disease (36.6% vs. 24.6%, P-value: 0.015), *T. tenax* (14.9% vs. 8%, P = 0.044) and *E. gingivalis* (24.6% vs. 19.4%, P = 0.246) in the case group were higher than in the control group. Also, after adjusting the effect of variables suspected of confounder, Compared to the control group, the odds ratio of gingivitis in people with infected all oral parasitic diseases, *T. tenax* and *E. gingivalis* were equal to 2.71 (95% CI: 1.50-4.88), 3.95 (95% CI: 1.78-8.76) and 1.46 (95% CI: 0.76-2.80), respectively.

**Conclusion**: This study showed a significant relationship between gingivitis and parasitic disease. Also, the coefficient of agreement between the two methods of Ttrichrome and Giemsa in diagnosing the *E. gingivalis* parasite was higher than *T. tenax*.

**Keywords**: *Trichomonas tenax*, *Entamoeba gingivalis*, gingivitis

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Poster

The design and formulation of ABZ-loaded nanostructured lipid carriers and assessment of their effect on hydatid cyst and prophylactic efficacy on secondary echinococcosis in mice

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**Background**: Cystic echinococcosis is one of the zoonotic helminthic diseases, which is caused by infection with the larval stage of the *Echinococcus granulosus*. The purpose of this study was to enhance the efficacy of albendazole (ABZ) on E. granulosus protoscoleces and metacestodes by preparing ABZ-loaded nanostructured lipid carriers (ABZ–NLCs).

**Methods:** ABZ–NLCs are prepared by the hot high-speed homogenization method. The particle size distribution, polydispersity index, and surface charges of the NLCs were measured using dynamic light scattering. The morphology of nanoparticles was investigated using transmission electron microscopy (TEM). *E. granulosus* protoscoleces and microcysts were treated in vitro with ABZ–NLCs and free ABZ at the final concentrations of 1, 5, and 10 µg/mL and the corresponding effects were examined by methylene blue dye exclusion test and TEM and scanning electron microscopy. In addition, chemoprophylactic treatment was performed on experimentally infected BALB/C mice.

**Results**: The NLCs were stable at 4 °C and 25 °C for one month with an average size of 114.67  $\pm$  10.1 nm. TEM showed spherical shape nanoparticles with size 60–150 nm. All protoscoleces incubated with the 10 µg/mL ABZ–NLCs were killed on day 18, while incubation with the 10 µg/mL of free ABZ killed all of them on day 27 post-incubation. In mice treated with ABZ–NLCs, a significant (P < 0.05) decrease was observed in weight (0.28  $\pm$  0.13 g) of cysts compared to the control group, yielding an efficacy of 92.45%.

**Conclusion**: The results clearly indicated that treatment of *E. granulosus* protoscoleces and metacestodes with ABZ–NLCs are significantly more efficient than treatment with free ABZ.

**Keywords**: *Echinococcus granulosus*, protoscoleces, echinococcosis, nanoparticles **Correspondence Email(s):** Hani@zums.ac.ir



The effect of chronic *Toxoplasma gondii* infection on the course of parkinson's disease in a mouse model

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**Background**: *Toxoplasma gondii* is a neuroinvasive protozoa pathogen that could manipulate its intermediate host's behavior. However, the possible link between *T. gondii* infection and the development of neurodegenerative disorders such as Parkinson's disease (PD) has been proposed, and we tested the hypothesis that in chronic toxoplasmosis neuroinflammation and molecular mediators potentiate.

**Methods:** To establish chronic toxoplasmosis by Tehran strain, cysts of *T. gondii* were injected intraperitoneally into BALB/c mice in Kerman, Iran in 2019. To induce the PD model, mice (BALB/c) were treated with Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The behavioral experiments such as anxiety and motor coordination were performed using the Open field and Rotarod tests. Additionally, we investigated the contribution of *Toxoplasma*-induced neuroinflammation, and behavioral-cognitive impairments in the PD mice model.

**Results**: Chronic toxoplasmosis caused PD-like symptoms and induced various behavioral changes in infected BALB/c mice. In *T. gondii* infected+MPTP treated group, *T. gondii* infection could potentiate PD in infected mice receiving MPTP and caused remarkable dysfunction in motor coordination and change in anxiety and depression-like behaviors similar or more severe than PD group.

**Conclusion**: Chronic *T. gondii* infection exacerbates pathological progression of PD in BALB/c mice brain by promoting neuroinflammation, and behavioral changes establishing.

**Keywords**: *Toxoplasma gondii*, methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), behavioral

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# Poster

The effect of fasting diet on experimental hydatidosis in BALB/c mice

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**Background**: Hydatid cyst is one of the important zoonotic diseases in Iran. In this study, the effect of fasting diet on hydatid cysts was investigated in infected BALB/c mice.

**Methods:** In this study, 30 BALB/c mice divided into two case and control groups. In the control group, the mice had enough water and food for 100 days, in the case group, the mice had water and food for one day and then had a fasting diet for two days, and only water available. The mice infected with 1500 protoscolices and kept under the normal conditions for 200 days. The mice were dissected, then the internal organs were examined for the presence of hydatid cysts and their number. Also, samples were taken from the infected tissues with cysts for histopathological examination.

**Results**: In the control group a total of 21 cysts, including 19 cysts in the liver and 2 cysts in the lung with a size of 1 to 5 mm were counted, while in the case group, three mice were infected with cysts and the number of cysts counted was three in size from one to three mm. The results of histopathological examination showed the similar tissue lesions around the cysts in both groups.

**Conclusion**: The results obtained in this study showed that fasting diet may be effective to decrease the number and size of hydatid cysts. However, more studies are needed to draw firm conclusions.

**Keywords**: Fasting diet, hydatid cyst, BALB/c

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The effect of hydatid cyst fluid with G1 and G5 genotypes on Th1/Th2 cytokine profile

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**Background**: Cystic echinococcosis (CE) is a chronic zoonotic disease caused by the larval stage of *Echinococcus granulosus*, which affects domestic and wild carnivores as the definitive host and ungulates as intermediate hosts. In intermediate hosts, both Th1 and Th2 cells are involved in the immune responses to CE.

**Methods:** Isolated spleen lymphocyte cells were treated with different concentrations (10%, 20%, 30%, 40%, and 50%) of hydatid cyst fluid (genotypes G1 and G5). The toxicity was determined by MTT. The expression level of cytokines IL2, IL4, IFN $\gamma$ , and TNF $\alpha$  was measured by Real Time-PCR.

**Results**: The results of the proliferation test showed that the affected concentrations of the cyst fluid were not toxic for the cells, and the examination of the cytokine levels showed that the changes in the expression levels of IL2, IFN $\gamma$ , and TNF $\alpha$  cytokines between the G1 and G5 groups were not significant, while the changes for IL4 is significant between two groups (*P* = 0.02).

**Conclusion**: Significant changes in Th2 (IL4) cytokine levels between two different groups G1 and G5 are probably due to the antigenic differences between these two groups.

Keywords: Hydatid cyst, cystic echinococcosis, IL2

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Poster

The effect of hydroalcoholic extract of *Quercus brantii* and *Artemisia aucheri boiss* against *Trichomonas vaginalis* in vitro

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**Background**: Trichomoniasis is the most common sexually transmitted protozoan infection worldwide. Metronidazole is widely considered as the drug of choice for treating of trichomoniasis but considering its potential side effects, we aimed to assess the therapeutic influences of hydro-alcoholic extracts of *Quercus brantii* and *Artemisia aucheri boiss* as alternative medications.

**Methods:** The trophozoites were cultured in TYI-S-33 medium at a density of 5x105 trophozoites/mL. Subseq uently, they were incubated with varying concentrations of the plant extracts (32, 64, 125, 250, 500, and 1,000 µg/mL) and metronidazole (16, 32, 64, 125, 250, and 500 µg/mL), as the positive control. The number of trophozoites in each well plate was quantified after 2, 4, 6, 24, 48, and 72 hours using trypan blue staining. Finally, the viability of the parasite was assessed by vital methylene blue staining.

**Results**: The hydro-alcoholic extracts of *Q. brantii* and *A. aucheri boiss* at concentrations of 125, 250, 500, and 1,000  $\mu$ g/mL demonstrated significant efficacy against the parasite. Our findings indicated that the minimum effective concentrations were 125  $\mu$ g/mL and hydro-alcoholic extracts of Q. brantii and A. aucheri boiss have the ability to effectively eliminate *T. vaginalis* after 48 and 72 hours of treatment.

**Conclusion**: The findings of the present study showed that hydroalcoholic extract of *Q. brantii* and *A. aucheri boiss* can induce death in *T. vaginalis*. However, further complementary in vivo studies are needed to assess the components of these plants in the treatment of *T. vaginalis*.

**Keywords**: *Trichomonas*, extract, *Quercus brantii*, *Artemisia aucheri boiss* 

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The effect of *Leishmania major* and *Leishmania* infantum antigens on A-375 human melanoma cell growth

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**Background**: Cancer is one of the main causes of death worldwide, which kills approximately 10 million people every year. Conventional treatments are not able to eliminate the tumor, so alternative methods are highly essential. Immunotherapy/biotherapy is a new approach to cancer treatment that increases the body's ability to fight disease.

**Methods:** A-375 cells were cultured in a DMEM medium. *Leishmania major* and *Leishmania infantum* parasites were cultured in RPMI 1640 medium. Crude antigens were prepared from parasites in the logarithmic phase to treat melanoma cells at different concentrations and times. Then cytotoxicity, cell cycle, and apoptotic changes were evaluated in A-375 cells compared to normal cells.

**Results**: The results of the cytotoxicity test showed a lower survival of A-375 cells treated with crude *L. major* and *L. infantum* antigens and DAC-treated cells compared to the human umbilical vein endothelial cells (HUVECs). The selective index (SI) for crude *L. major* and *L. infantum* antigens and DAC was 1.95, 8.45, and 2.80 respectively. The apoptotic ratio of melanoma cells treated with crude extracts is significantly higher compared to the normal cells. Cell cycle evaluation showed that treatment with the crude extracts caused a significant improvement in the S phases, which affects the rate of cell division.

**Conclusion**: The *L. major* and *L. infantum* crude antigens can be effective in inducing apoptosis, cytotoxicity, and cell cycle arresting in melanoma cancer cell lines. Further in vivo and clinical studies are crucial to explore exactly the impact of these *Leishmania* antigens against is needed.

**Keywords**: Leishmaniasis, melanoma, cancer, apoptosis, cytotoxicity

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Poster

The effect of paromomycin sulfate loaded in mesoporous silica nanoparticles on cutaneous ulcers induced by *Leishmania major* in animal model

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**Background**: Considering the anti-parasitic properties of paromomycin and the unique futures of mesoporous silica nanoparticles for drug delivery, we determined the effect of paromomycin sulfate loaded in mesoporous silica nanoparticles (PM10%-MSN) on cutaneous ulcers induced by *Leishmania major* in animal model.

**Methods:** After making and assessment of size and quality the PM10%-MSN, the IC50 of the nano-drug on healthy macrophages and promastigote were evaluated by MTT method. Then 30 female Inbred BALB/c mice were infected by about 3-5 thousands promastigotes and after creating ulcers, the mice were divided into 5 groups (Negative control, Positive control, MSN treatment, paromomycin treatment (PM) and PM10%-MSN treatment). The mice were treated daily for 21 days, and the area of wounds and parasite load were measured.

**Results**: The results showed a significant decrease from 66.38 to 53.12 mm2 in the average wound area of the mice treated with PM10%-MSN (P = 0.035). However, there was no significant difference between wound area of all groups in the first to fourth weeks, but there was a significant difference between the negative control and PM10%-MSN groups in the fifth week(P = 0.041). The parasite load decreased in PM-10%-MSN group in comparison with negative control group.

**Conclusion**: The results of this study indicate that the prepared nano-drug showed good anti-leishmanial properties in vitro. The paromomycin sulfate ointment loaded in the mesoporous silica nanoparticles (PM10%-MSN), has significantly improved the size of the wound and reduced the parasitic load of *L. major*, and it can be used as an

**Keywords**: Cutaneous leishmaniasis, mesoporous silica, paromomycin

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# Poster

The effect of prednisolone and mometasone on the improvement of ulcers caused by *Leishmania major* in mice

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**Background**: Currently, no effective leishmaniasis vaccine exists, making chemotherapy the primary treatment option. However, standard treatments can be toxic, expensive, and face resistance. This study aimed to assess how prednisolone and mometasone affects the healing of cutaneous leishmaniasis in BALB/c mice.

**Methods:** This experimental study was conducted by using prednisolone and mometasone drugs on 60 BALB/c female mice. Promastigote form of *Leishmania major* obtained from culture medium was injected into the right footpad of the mice. After ulcer formation, the mice were divided into 6 treatment groups: prednisolone, prednisolone with amphotericin B, mometasone, mometasone with amphotericin B, positive control (amphotericin B), and negative control (PBS). The treatment period was 28 days, and at the end of each treatment week, the ulcer diameter was measured.

**Results**: Significant reductions in ulcer size and parasite burden in the spleen were observed in the two treatment groups with prednisolone and prednisolone in combination with amphotericin B at the end of the treatment period (P < 0.050).

**Conclusion**: Based on the results of the present study, it appears that glucocorticoids accelerate healing by regulating immune pathways, preventing ulcer chronicity and severity.

Keywords: Mometasone, prednisolone, cutaneous leishmaniasis

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Poster

The effect of *Rubbia tinctorium* extract on cutaneous leishmaniasis in BALB/c Mice

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**Background**: Cutaneous leishmaniasis is self-healing while visceral leishmaniasis is fatal. Efforts have been made since more than a century ago to control and treat the disease, but appropriate vaccines, drugs and pesticides have not yet been prepared. *Rubbia tinctorium* has been used traditionally in medicine throughout history.

**Methods:** *Rubbia tinctorium* extracts with 40%, 60% and 80% concentrations were prepared. Then, the BALB/c mice were infected with *Leishmania* (*L.*) *major* [MRHO/IR/75/ER]. Soon after the ulcer started to appear in the early stage, one dose of the herbal medicine with 40%, 60% and 80% concentration on honey base was used to treat the ulcer and dosing continued till the death of the mice of negative control group occurred.

**Results**: The mean weight of the mice that received 40% concentration of *R. tinctorium* extract showed a statistically significant difference compared to the mean weight of the mice receiving 60% concentration of *R. tinctorium* extract (P = 0.001).

**Conclusion**: When the weight decreased and lesion size of the cutaneous leishmaniasis widened, the animal would die. As a whole, in the all of the time, the mice that received 40%, 60% and 80% *R. tinctorium* extract their lesion were wet, without secondary infection and without necrosis.

**Keywords**: Cutaneous leishmaniasis, *Rubbia tinctorium*, extract,

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### Poster

The effect of sanguinarine on the hydatid cyst protoscoleces in vitro and in silico

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Background: Our study aimed to investigate the scolicidal effects of sanguinarine on hydatid cyst protoscoleces (PSCs) through in vitro and in silico experiments. Sanguinarine's active sites were utilized to dock various targets.

Methods: The docking process and visualization of interactions were carried out using AutoDock Vina and Discovery Studio Visualizer. The binding energy was calculated and subsequently compared in kcal/mol. PSCs were extracted from the hydatid cysts and underwent thorough washing. The binding energy was computed and compared in units of kcal/mol. PSCs were collected from the hydatid cysts and subsequently washed. The sediments of PSCs were then exposed to different concentrations (50, 25, 12, 6, 3, and 1  $\mu$ g/mL) of sanguinarine. The viability test was ultimately assessed using a 4% Trypan blue solution. Levels of MDA, SOD, GSH, measured

Results: Among the receptors, acetylcholinesterase was identified as an excellent target, achieving a Vina score of -11.8. Sanguinarine exhibited remarkable scolicidal effects after 12, 24, and 48 hours. Furthermore, within the first hour of drug exposure, there was a significant increase in caspase-3 activity and MDA level. Conversely, the levels of GSH and GPx experienced a notable reduction after 12, 24, and 48 hours (*P* < 0.05).

**Conclusion**: The study's results indicate that sanguinarine exhibits strong scolicidal effects both in vitro and in silico. This suggests that sanguinarine could potentially serve as a safe and innovative therapeutic option for treating cystic echinococcosis. Nevertheless, further research is necessary to validate these findings in a clinical setting.

Keywords: Sanguinarine, scolicidal, docking

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Poster

The effect of toxoplasmosis on valproic acid-induced animal model of autism, an in vivo study

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**Background**: Toxoplasmosis is a disease caused by infection with a type of coccidial protozoan parasite called Toxoplasma gondii. The relationship between toxoplasmosis and cognitive disorders in neurodegenerative diseases has been proven. There is also evidence that children born to Toxoplasma-infected mothers are more likely to develop autism.

Methods: In this study, male offspring of Toxoplasma-infected pregnant BALB/c mice were induced with autism using valproic acid, and their social behaviors, learning, and memory were evaluated. Chronic toxoplasmosis was induced in BALB/c mice by injecting cyst form of T. gondii intraperitoneally. To trigger autism, 600 mg/kg of valproic acid was also injected intraperitoneally into pregnant mice on the 12.5th day of their pregnancy. Male offspring were subjected to behavioral experiments such as social interaction, novel object recognition, and passive avoidance tasks when they were 50 days old.

**Results**: *Toxoplasma* and valproic acid during the embryonic period caused social communication deficits and disrupted recognition memory and avoidance memory in offspring. Our findings showed that administering valproic acid to Toxoplasma-infected mothers exacerbates cognitive disorders in their offspring

Conclusion: Further research is needed to understand the underlying mechanisms linking maternal T. gondii and autism. Our findings suggest that Toxoplasma infection in pregnant mothers exacerbates cognitive impairments in the animal model of autism.

Keywords: Autism, memory, social interactions, Toxoplasma

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The effectiveness of nano-ebulin isolated from Sambucus ebulus inside phospholipid capsule on Leishmania major parasite in vitro and in vivo

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**Background**: Leishmaniasis is one of the most important parasitic diseases in tropical and semi-tropical regions. Considering the increasing reports of resistance to glucantim, a common treatment drug, it seems necessary to study and investigate finding a safe and natural alternative drug for the treatment of leishmaniasis.

**Methods:** The purpose of this study is to investigate the effect of nano-ebulin isolated from *Sambucus ebulus* (*S. ebulus*) inside phospholipid capsule on *L. major* parasite. The standard strain of *Leishmania major* (*L. major*) parasites were and cultured in RPMI-1640 medium at 24°C. S. ebulus was extracted using maceration method and ebulin was extracted by column chromatography technique. Ebulin nanoparticles were prepared using ionic gelation method. First, the effect of ebulin nanoparticles on promastigotes stage was investigated and in the next step, the drugs was used to treat the wound caused by cutaneous leishmaniasis.

**Results**: The results of this study showed that Ebulin nanoparticles were effective at all concentrations on promastigotes of *L. major* and even at a concentration of 800  $\mu$ g/mL it was more effective than glucantim. In in vivo conditions, Ebulin nanoparticles at concentrations of 200, 400 and 800  $\mu$ g/mL had a better effect than amphotericin B as a positive control group, but the difference was significant only at concentrations of 400 and 800  $\mu$ g/mL.

**Conclusion**: Considering the effectiveness of ebulin nanoparticles on the *L. major* parasite and the better effect than the positive controls, it can be concluded that the above compounds can be suitable candidates for the treatment of leishmaniasis, of course, after conducting additional studies.

Keywords: Leishmania major, Ebulin, Sambucus ebulus

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Poster

The effects of hydroalcoholic extract of *Calendula* officinalis flower on *Leishmania major* Ulcer in Balb/c mice

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**Background**: *Calendula officinalis* plant increasingly used as an anti-inflammatory for external use and wound healing. The aim of this study was to evaluate the effect of hydroalcoholic extract of Calendula officinalis on *Leishmania major* ulcer in BALB/c mice

**Methods:** This experimental invitro study was performed on Balb/c mice infected with *L. major* parasite. After preparing Calendula officinalis extract, the treatment was performed on the wound daily for three weeks. Wound diameters in rats were measured every other day with a caliper for up to three weeks.

**Results**: According to the results of this study, the mean wound size in the group treated with *Calendula officinalis* was significantly lower than the control group. Also, the survival rate in patients treated with *Calendula officinalis* was higher compared to the control group.

**Conclusion**: It seems that hydroalcoholic extracts of *Calendula officinalis* flower is effective in treating patients with leishmaniasis ulcers. Therefore, *Calendula officinalis* extract seems to be a suitable treatment for wound patients due to its effectiveness, low cost and low side effects.

**Keywords**: Leishmaniasis, *Calendula officinalis*, survival

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The effects of miltefosine on the *Toxoplasma gondii* RH strain and the evaluation of cytotoxic and apoptotic in vitro

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**Background**: Our study aimed to examine the effects of miltefosine on the RH strain of *Toxoplasma gondii* using a range of techniques, focusing on cytotoxicity and apoptosis.

**Methods:** The cytotoxicity test involved the addition of miltefosine to *T. gondii* tachyzoites; control tachyzoites were treated with PBS and MTT assay was carried out on each suspension. To assess the Th1 the Th1-type immune responses, the levels of IFN- $\gamma$  and nitric oxide (NO) in the mice's serum were examined following the injection of tachyzoites and miltefosine. The flow cytometry technique was utilized on *T. gondii* tachyzoites that were challenged with IC50 and IC90 doses of miltefosine, as well as on unchallenged cells.

**Results**: The concentrations of miltefosine at 256, 64, 32, and 16 µg were able to eliminate over 50% of viable *T. gondii* tachyzoites effectively. The group of mice infected with *T. gondii* and treated with miltefosine showed a significant increase in the production of IFN- $\gamma$  compared to the other groups (P < 0.001). Additionally, a notable difference in the levels of inducible NO synthase was observed between the experimental and control groups (P < 0.05). Flow cytometry analysis revealed that the rate of apoptosis in tachyzoites incubated with miltefosine was dependent on the concentration, while the rate of necrosis did not show any significant difference.

**Conclusion**: Miltefosine has the potential to be a favorable candidate for the novel treatment of toxoplasmosis.

**Keywords**: Interferon-gamma, miltefosine, nitric oxide, tachyzoite

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Poster

The effects of Tio2 nanoparticles with glucantime in infected BALB/c mice with cutaneous leishmaniasis, a histopathologic investigation

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**Background**: Leishmaniasis is one of the neglected tropical diseases in the world. The drug resistance and toxicity and the high cost of the drugs with lack of novel anti-leishmanial drugs highlight the need to find newer drug.

**Methods:** The treatment regimen for each group of mice was once a day for 28 days as follows: three groups received nanoparticle + glucantime at dosages of 100, 50, 25 mg/mL, three groups received  $TiO_2$  + chitosan at dosages of 100, 50, 25 mg/mL, a positive control group received 100 mg/mL of glucantime, a group received 100 mg/mL of PBS as negative control group. After preparing the tissue sections of liver and spleen, the samples were stained with hematoxylin eosin and evaluated using a light microscope to find histopathological changes.

**Results**: Liver from treated mice with nanoparticle + glucantime showed normal cellular architecture without dilatation of central vein like positive control group, while the group received  $TiO_2$  + chitosan and negative control mice showed focal necrosis and dilatation of the central vein which is a sign of inflammation, as well as macrophages accumulations, necrosis of hepatocytes. In spleen, giant histiocysts with parasites were observed in the red pulp of the group received  $TiO_2$  + chitosan and negative control while this observation was not seen in the group which treated mice with nanoparticle + glucantime.

**Conclusion**: Cutaneous leishmaniasis can cause histopathological changes in the spleen and liver of the infected BALB/c mice. The results proved the anti-leishmanial activity of nanoparticles.

**Keywords**: Cutaneous leishmaniasis, histopathologic, Tio<sub>2</sub> nanoparticles

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#### Poster

The elimination trend of zoonotic cutaneous leishmaniasis in southeastern Iran: a longitudinal study from 1991 to 2021

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**Background**: Similar to other communicable diseases, leishmaniasis is a climate-sensitive illness affected by temperature, precipitation, and humidity. This study examined the trends in the epidemiology of cutaneous leishmaniasis (CL) over 30 years. It explored its relationship with the increasing urbanization and changes in the climate, environment, and agricultural practice.

**Methods:** This population-based prospective longitudinal study was conducted between 1991 and 2021 in a well-defined CL focus in Bam district, southeastern Iran. A robust health clinic and health surveillance system were responsible for the ongoing systematic documentation, detection, identification, and management of CL cases. The exponential smoothing method via the state space model was applied in the univariate time series. The TTR, smooth, and forecast packages were used in R software. Landsat satellite images from 1991, 2001, 2011, and 2021 were employed in the physical development.

**Results**: During this period, the temperature increased while the rainfall and humidity reduced. The findings showed a downward trend in the standardized drought index. Based on these results the forecast of this trend in the coming years is expected that day by day the risk of drought will threaten all creatures more than ever. Also, the findings showed that climate warming and ecological changes profoundly affected the area's agricultural patterns and topographical features. Moreover, the last three decades witnessed an elimination trend for zoonotic CL (ZCL) and the predominance of anthroponotic CL (ACL).

**Conclusion**: The current findings showed that the critical factors in the predominance and intensive spread of ACL and elimination of ZCL were rising temperature, drought, migration, unplanned urbanization, earthquake, and agrarian reform. Hence, robust health infrastructures, sustained financial support, and evidencebased research studies are crucial to control and eliminate the disease.

Keywords: Elimination, cutaneous leishmaniasis, longitudinal study

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# Poster

The frequency of the *Haemoproteus* infection in domestic pigeons in Mashhad area, Iran

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**Background**: *Haemoproteus* is a parasite that infects pigeons and doves and is widespread in tropical and subtropical areas. The protozoa are intracellular parasites that infect erythrocytes. While the pathogenicity of this parasite is generally low, severe infections in young pigeons can lead to significant mortality.

**Methods:** The current epidemiological survey examined the frequency of pigeon *Haemoproteus* spp. in eight distinct regions in Mashhad, northeastern Iran.121 blood samples were collected from domestic pigeons in Mashhad, Iran, from January to February 2024. Thin smears were prepared from wing vein; the blood smears were stained by Giemsa method and then examined using light microscope.

**Results**: In this this study, *Hemoproteus* infection was microscopically detected in 69.42% (84/121) of blood smears.

**Conclusion**: Our findings reveal a high frequency of *Haemoproteus* spp. infection in pigeons of Mashhad area. Further parasitological and molecular studies with a larger sample size are needed to determine the prevalence of infection and its health significance in pigeons of this city.

**Keywords**: *Haemoproteus*, pigeon, epidemiological survey, Iran

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The immune response against *Toxoplasma gondii* in BALB/c mice induced by nanoliposomes containing Imiquimod adjuvant against toxoplasmosis

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**Background**: Toxoplasmosis is a common infection worldwide caused by *Toxoplasma gondii*, an intracellular parasite. In the present study, DSPC liposome with Imiquimod Adjuvant encapsulated in the liposome with tactile antigens of *T. gondii* is used, and the immunity level obtained from vaccination and resistance to toxoplasmosis is evaluated.

**Methods:** In the present research, a nano-liposomal vaccine containing soluble antigens (SA) was designed to evaluate the immunity and protective efficacy against *T. gondii* infection. Soluble antigens (SA) were achieved from tachyzoites, encapsulated in the liposome, and investigated via scanning electron microscope. Three times with 2-week intervals, BALB/c mice were immunized subcutaneously with different formulations. The level of protection against infection was assessed through the percent survival survey of BALB/c mice after challenge with tachyzoites of *T. gondii* RH strain; also, the type of generated immune response was determined by evaluating the generation of cytokine (IFN- $\gamma$ , IL-4) and IgG isotypes.

**Results**: The immunization with liposome DSPC + SA and liposome DSPC + Imiquimod + SA induced a substantial increase in anti-*Toxoplasma* IgG antibody as compared to the PBS group (P < 0.05). The IgG2a and IFN- $\gamma$  secretion highest levels were seen with liposome DSPC + Imiquimod + SA more than the control group (P < 0.01) and (P < 0.0001), respectively. After challenge with tachyzoites, less mortality was detected in the immunized mice by liposome DSPC + Imiquimod + SA that was meaningfully different (P < 0.01) in comparison to other groups.

**Conclusion**: Vaccination with liposome DSPC + Imiquimod + SA showed more survival rate and cellular immune reaction against *T. gondii*.

Keywords: Toxoplasmosis, liposome, imiquimod

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Poster

The impact of poor treatment adherence in creating unresponsive cases of cutaneous *Leishmania*sis: A casecontrol study

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**Background**: One of the overlooked factors in the treatment outcome of cutaneous leishmaniasis (CL) is poor treatment adherence (PTA). This study is designed to explore the impact of PTA in the induction of unresponsiveness to treatment in cases with anthroponotic CL (ACL) comparing conventional statistical modalities and machine learning analyses.

**Methods:** This investigation was carried out as case-control research in Kerman district, Kerman province, between February 2020 and October 2021. In total, 190 cases consisting of 50 unresponsive patients (case group), and 140 responsive patients (control group) with ACL were randomly selected. The data collecting form that included 25 queries (Q) was recorded for each case and analyzed by R software and genetic algorithm (GA) approaches.

**Results**: Complex treatment regimens (Q11), cultural and lay views about the disease and therapy (Q8), life stress, hopelessness and negative feelings (Q22), adverse effects of treatment (Q13), and long duration of the lesion (Q12) were the most prevalent significant variables that inhibited effective treatment adherence by the two methods, in decreasing order of significance. In the inherent algorithm approach, similar to the statistical approach, the most significant feature was complex treatment regimens (Q11).

**Conclusion:** Providing necessary knowledge about ACL and treatment of patients with chronic diseases and patients with misconceptions about chemical drugs are vital issues directly associated with the disease's unresponsiveness. Early detection, efforts to minimize side effects and giving hope to patients with anxiety can help patients adhere to the treatment.

**Keywords**: Cutaneous leishmaniasis, poor adherence, unresponsiveness

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Poster

# The molecular frequency and mouse bioassay examination of *Neospora caninum* in bovine aborted fetous

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**Background**: *Neospora caninum* is an important agent of abortion and reproductive loss in dairy cattle. The objective study was to detect *N.caninum* in bovine aborted fetous by molecular and mouse bioassay examination.

**Methods:** During 2019-2022, 121 bovine aborted fetuses were collected from dairy farms in Khorasan Razavi province. The fetal brain samples were screened for detection of the parasite DNA by PCR. In addition, a portion of PCR-positive brain tissue was homogenized and inoculated in five Balb-c mice. All mice were sacrificed post six-week inoculation and examined by serology, microscopy, and PCR methods. The brains of infected mice were homogenized and the mouse bioassay was repeated two times.

**Results**: The *N. caninum* DNA was detected in 19.8% (n = 24) brain samples of bovine aborted fetuses. All inoculated mice were seronegative without clinical signs and cysts in the brain after two times bioassays, although three brain samples were PCR-positive.

**Conclusion**: The results indicated that *Neospora* infection is common in dairy cattle in the Mashhad area and isolated Neospora has low pathogenicity in BALB/c mice.

**Keywords**: BALB/c mice, *Neospora*, PCR

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Poster

The molecular prevalence of *Cryptosporidium* spp. in the residents of Roudehen, Tehran province

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**Background**: *Cryptosporidium* species are zoonotic intestinal protozoa that cause infections in humans. An accurate assessment of this parasite prevalence plays an essential role in the prevention, control, and proper treatment of cryptosporidiosis in patients. Thus, we investigate the molecular prevalence of *Cryptosporidium* spp. among the residents of Roudehen, Tehran province.

**Methods:** Five hundred sixty-one triple fecal samples were collected during a cluster multi-stage sampling method. Molecular detection of *Cryptosporidium* spp. was performed by Nested-PCR of the small subunit ribosomal RNA (rRNA) gene.

**Results**: *Cryptosporidium* spp. was detected in 11 (2.6%) individuals by 18s rRNA gene amplification. The prevalence of *Cryptosporidium* spp. in males was higher than in females (4.3%). The highest prevalence of infection was seen in the 31-45-year-old group of participants (4.4%). The results showed a statistically significant relationship between cryptosporidiosis and gender (aOR = 4.3, 95% CI: 1.1-18.3). The male gender was considered a risk factor for *Cryptosporidium* infection.

**Conclusion**: The results showed that *Cryptosporidium* is circulated in residents of Roudehen. Because of the direct transmission of this parasite, individual health education can play an essential role in improving health and preventing the risk of cryptosporidiosis transmission to people living in this area.

Keywords: Cryptosporidium, nested-PCR, Roudehen

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The parasitic infection distribution among patient referred to therapeutic centers of Babol during 5 years

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**Background**: Intestinal parasitic infections are one of the significant concerns in hygiene and health care systems in underdeveloped and developing countries and their high prevalence is noticeable. These infections are caused by protoazoan and helminthes parasites. Among parasitic gastrointestinal infections, protozoan parasitic infections are more common than helminthic infections.

**Methods:** The cross-sectional study was carried out from January 2019 to January 2024. 6962 fecal samples were collected. The probable parasitic infections in the stool samples were investigated by microscopic examination, and direct techniques such as saline and iodine wet mounts and concentration technique were applied.

**Results**: Generally, parasitic infections (both helminthic and protozoan parasites) were detected in 680 samples out of 6962 tested samples which concludes 10.23% of referred samples. Overall, the prevalence of protozoan parasitic infection was higher than helminthic infection. Although *Entamoeba histolytica*/dispar (3.07%) and *Giardia lamblia* (1.1%) were two most observed protozoan parasites and *Strangyloides stercoralis* larva was the most abundant helminthic parasite among all none-protozoan parasites.

**Conclusion**: Gastrointestinal parasitic infections especially protozoan intestinal parasites are distributed all around the world and are mainly affect people by oral-fecal transmission which highlights the importance of environmental hygiene. Therefore, the understanding of parasitic infections distribution is important to elevate the health levels of the society in under-developed and developing countries.

Keywords: Parasitic infection, protozoa, helminthes

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Poster

The presence of *Trichomonas vaginalis* virus (TVV) in *Trichomonas vaginalis* isolates, Systematic Review

Katayoun Zahedpour <sup>1</sup> @, Mahmoudreza Behravan <sup>2</sup> ©

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**Background**: *Trichomonas vaginalis* is the causative agent of trichomoniasis, which is the most common non-viral sexually transmitted disease. *T. vaginalis* may be infected by a dsRNA virus called *T. vaginalis* virus (TVV). TVV is closely associated with the Golgi complex and is transmitted vertically.

**Methods:** In this review study, articles related to TVV were reviewed in various databases of Web of science, Pubmed, Scopus, Google Scholar and etc.

**Results**: In one study, it was demonstrated that out of 82 isolates, 29 of them were infected with TVV1, TVV2, and TVV3. Another study conducted at a clinic found that 21 out of 28 isolates were infected with T. vaginalis. An article from Italy showed that out of 48 T. vaginalis isolates, approximately half of them tested positive for at least one TVV strain, with TVV2 being the most prevalent. Yet another study revealed that out of 110 women, 40 of them had positive T. vaginalis samples, with five of them containing TVV2 and the remaining three being infected with TVV4.

**Conclusion**: The results privide an insight into the distribution of symbiont in *T. vaginalis* isolates. The relationship between TVV and *T. vaginalis* may be commensal.

**Keywords**: Trichomoniasis, *Trichomonas vaginalis*, TVV

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The prevalence and associated factors of head lice infestation in primary school children in Ahvaz county, southwestern Iran

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**Background**: Head lice are the most common type, especially in the age group of 3 to 11 years. They mainly affect schoolchildren from various economic and social groups, and are more prevalent in girls than boys. The present research was carried out to investigate the prevalence of head lice and related.

**Methods:** The sample size was 5930 students that was determined by using a single population proportion formula. Samples were selected by a multistep, classification random sampling strategy. The collected data was evaluated using SPSS software. These data were compared using a *Chisquare* analysis.

**Results**: The overall head lice infestation rate in the studied population was 26.3%. The prevalence rate was 15.2% in the boys and 37.9% in the girls, while the rate of infestation was 25.1% in the urban areas and 36.9% in the villages. Also, a statistical significant relationship was observed between Pediculosis capitis and gender, access to tap water, sleeping in the common bedroom, use common bedding, presence health educator in school, access to primary health services, knowledge about head lice, performing personal hygiene and etc.

**Conclusion**: The high prevalence of head lice among students in the majority of primary schools in Ahvaz county is attributable to inadequate access to health educators, sanitation facilities, inattention to personal health, and other related factors. Resolving such a problem requires the cooperation of various bodies such.

 Keywords: Epidemiology, Pediculus capitis
 Intervention of the second second

Poster

The prevalence of *Sarcocystis* sp in cattle in Shahrekord county by parasitological methods in 2021-2022

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**Background**: *Sarcocystis* is a widely distributed obligate intracellular protozoan parasite of the phylum Apicomplexa that infects a wide range of hosts including mammals, birds and reptiles. Some species of *Sarcocystis* are zoonotic between humans and animals.

**Methods:** In this descriptive cross-sectional study, a total of 130 samples, including diaphragm tissues from cows and calves were collected from butcher shops in Shahrekord county during the 2021-2022. The samples were examined using parasitological methods, including macroscopic observation (naked eye examination), histological examination (Muscle squash), peptic digestion method, then the results were analyzed using SPSS software.

**Results**: In the macroscopic investigation was not observed any cyst but the dub smear and histological examinations showed that 26.9% and 70% of the meat were infected with *Sarcocystis* sp, respectively.

**Conclusion**: The relatively high prevalence of microscopic cysts of *Sarcocystis* in the meat indicates a high level of contamination in the meat-producing animals. Therefore, ensuring the thorough cooking of meat and meat products and increasing awareness of prevention of contamination will be effective.

**Keywords**: *Sarcocystis*, cattle, meat, parasitological methods

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The prevalence of *Toxoplasma gondii* in aborted livestock fetuses from northeast Iran, from molecoular to serological examinations

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**Background**: Toxoplasmosis is a disease that can cause abortions in both humans and herbivores, resulting in significant financial and quality of life losses. This study aims to determine the prevalence of toxoplasmosis in aborted fetuses using serological and molecular assays, and to identify the genetic make-up of the isolates obtained.

**Methods:** Researchers used serological and molecular methods to study aborted fetuses from Bojnourd city in North Khorasan province, Iran, including 52 sheep and 16 cows. The B1 gene was detected using Nested PCR, which revealed parasite DNA in the brain tissues of the fetuses. To determine the genotype of *T. gondii*, the PCR-RFLP method of the GRA6 gene was employed.

**Results**: Among 68 fetuses that were aborted, 16.1% showed the presence of anti-*T. gondii* IgG. Out of these, 11.7% were found in bovine fetuses and 4.4% in ovine fetuses. In addition, two (2.94%) samples from ovine tested positive for anti-*T. gondii* IgM. Our PCR analysis identified parasite DNA in two cases (2.94%) out of 11 IgG-positive samples. All obtained isolates belong to type I of *T. gondii*.

**Conclusion**: It has been observed that infection with Type I of *T. gondii* during the neonatal period may contribute to abortion and economic losses in livestock farming in our studied region. In order to gain a better understanding of the molecular epidemiology and genotypes of *T. gondii* associated with abortion.

Keywords: *Toxoplasma*, abortion, molecular, genotyping, ruminant

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Poster

The role of *Leishmania major* lysate, excretory/secretory antigens and a combination of them on the expression of IFN- $\gamma$  and IL-4 and mice surveillance

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**Background**: Leishmaniasis has a complex of clinical symptoms, it spreads to humans through the bite of a mosquito, despite the passage of more than a century of efforts made in the field of disease control and treatment, a suitable vaccine, medicine and insecticide have not yet been prepared for it.

**Methods:** *Leishmania* (L.) major [MRHO/IR/75/ER] was cultured and excretory-purified and lysed antigen prepared. After injecting antigens with adjuvant in twice to mice of BALB/c breed, monitoring and recording the weight and size of the wound diameter of the mice continued until the death of the last mouse from the negative group, and finally, by dissecting the mice, the width of the spleen of each group was measured and recorded.

**Results**: The average weight of mice in the negative group showed no significant difference with the average weight of mice receiving adjuvant (P > 0.05), but it showed a significant difference with the average weight of mice receiving excretory-secretory antigens, lysed and combined (P < 0.05). The mean size of the wound diameter of the mice in the negative group showed a significant difference with the mean size of the wound of the group that received excretory-secretory and lysed antigens (P < 0.05). The size of the spleen of mice in the negative group showed no significant difference with any of the groups receiving *Leishmania* antigen (P > 0.05).

**Conclusion**: Investigations should be done at the cellular and even molecular level, because the greater the immunogenic and protective components are exposed to the human immune system, the more tangible the results will be, and the antigen used should be a conjugation of adjuvants, immunogens, and macrophage proliferators.

Keywords: Excretory-secretory Ag, lysate Ag

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5th International & 12th National Congress of Parasitology and Parasitic Diseases of Iran	
NIZOPA	Alborz University of Medical, Sciences, Karaj, Iran
	May 21 - 23, 2024
Destas	Destar
Poster	Poster
The role of tissue inhibitor of metalloproteinase-1 and 2 in <i>Echinococcus granulosus</i> senso lato-induced human hepatic fibrosis	The role of toxoplasmosis in psychiatric disorders: A case- control study in Gorgan, Iran
Azadeh Hasanzadeh <sup>1</sup> ©, Abdollah Rafiei <sup>1</sup> , Mohammad Kazemi <sup>2</sup> , Molouk Beiromvand <sup>1</sup> ©, Amin Bahreini <sup>3</sup> , Hossein Khanahmad <sup>2</sup>	Mitra Sharbatkhori <sup>1</sup> © Ø, Hamidreza Zakerzadeh Khoshroodi <sup>2</sup> , Najmeh Shahini <sup>3</sup> , Farideh Tohidi <sup>1</sup> , Rasool Mohammadi <sup>4</sup> , Aieneh Hajieh Pangh <sup>2</sup>
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<b>Background</b> : The main mechanism underlying hepatic fibrosis is the imbalance between tissue Matrix Metalloproteinases (MMPs) and Tissue Inhibitors of Metalloproteinases (TIMPs). This study aimed to investigate the potential role of TIMP-1 and TIMP-2 in the process of hepatic fibrosis caused by <i>Echinococcus granulosus</i> senso lato ( <i>E. granulosus</i> senso lata).	<b>Background</b> : Toxoplasmosis, is the most common parasitic disease in the world, caused by an intracellular parasite called <i>Toxoplasma gondii</i> . The aim of this study was to investigate the prevalence of IgM and IgG antibodies against <i>Toxoplasma</i> in patients hospitalized in the psychiatric department compare to healthy people in Gorgan in 2023.
<b>Methods:</b> The expressions levels of TIMP-1 and TIMP-2 mRNAs were evaluated in fibrotic and normal hepatic tissues of 30 patients with cystic echinococcosis (CE) using qRT-PCR. Moreover, their serum levels of TIMP-1 were assessed before CE cyst removal and 6 months after surgery using ELISA.	<b>Methods:</b> This was a case-control study. The case group consist of 100 patients hospitalized in the psychiatry department of 5Azar Hospital in Gorgan, and the control group consist of 100 healthy individuals referred to the Deziani Clinic laboratory. A five mL blood sample was taken from eachparticipant. Then, the presence
<b>Results</b> : The qRT-PCR results showed that the expression levels of TIMP-1 and TIMP-2 mRNAs were significantly higher in the fibrotic hepatic tissue compared to the normal liver tissue, in a way that the TIMP-1 and TIMP-2 mRNA expression levels were 19.07 and 6.58 folds higher in the fibrotic tissue compared to the normal liver tissue. Among these TIMPs, TIMP-1 exhibited the higher area under the curve (AUC) value for predicting liver fibrosis	of anti- <i>Toxoplasma</i> IgG and IgM was detected in serum samples by ELISA method. After entering the data into SPSS software version 22, descriptive statistics of the central and dispersion indices were reported, and independent t-test, chi-square and logistic regression were used to analyze the data at a significance level of 0.05.
However, we could not find a significant difference in the serum levels of TIMP-1 before and after the cyst removal procedure ( $P = 0.48$ ).	<b>Results</b> : The frequency of IgG and IgM antibodies in the case and control groups was 39% and 47%, respectively, with no significant statistical difference. A significant relationship was observed between toxoplasmosis infection and marital status, place of
<b>Conclusion</b> : our study showed that the significant overexpression of both TIMP mRNAs in the fibrotic liver tissue of the CE patients may be due to the increased expression of MMPs in the peri-cystic	residence, ethnicity, occupation, education level, contact with cats, consumption of raw vegetables, and contact with soil ( $P < 0.05$ ).
tissue. However, we could not find a significant difference in the pre- and post-operative TIMP-1 levels.	<b>Conclusion</b> : The results of this study suggest that toxoplasmosis is not related to psychiatric disorders, but has different risk factors among psychiatric patients and healthy controls in Gorgan, Iran.
Keywords: Cystic echinococcosis, TIMP-1, TIMP-2	This may have implications for the prevention and treatment of toxoplasmosis.
Correspondence Email(s): beiromvandm@gmail.com	Keywords: Psychiatric disorders, Toxoplasma gondii, toxoplasmosis
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The status of cutaneous leishmaniasis in Nahavand county, Hamadan province, during 2014-2022

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**Background**: Cutaneous leishmaniasis is the most common form of leishmaniasis affecting humans and animals. This disease is considered as one of the health problems in some provinces of Iran. The aim of present study was to investigate the status of cutaneous leishmaniasis in Nahavand county, Hamadan province, during 2014-2022.

**Methods:** Demographic data of 110 cutaneous leishmaniasis patients during the 2014 to 2022 whom referred to the health center of Nahavand county was obtained. This information included gender, age, place of residence, season of ulcer occurrence, involved organs, jobs, number and size of lesions, travel history and treatment methods of patients. SPSS version 26 software was used for data analysis. In order to investigate the relationship between qualitative variables, *Chi-square* test was used and the probability value less than 0.05 (P < 0.05) was considered as a significant level.

**Results**: 89.1% of patients had traveling history to endemic areas, while 10.9% did not. The majority of patients traveled to Mehran city (37.3%), followed by Ahvaz and Dehlaran (7.3% and 5.5%). The most of patients were men, with the highest frequency found in of 41-50 age group. 53% of patients have urban life, 44.5% lived in villages, and 1.8% were nomads. The January and February recorded the highest referral and leg ulcers being the most common condition (40%). Additionally, labor jobs (34.5%) and treatment with topical antimony (71.8%) were more prevalent. *Chi-square* statistical analysis of all variables was significant (P < 0.001).

**Conclusion**: The results of this study showed there are cutaneous leishmaniasis patients without travel history in Nahavand county. On the other hand, according to the report of potential cutaneous leishmaniasis vectors in the region, timely identification and treatment of new patients is necessary for prevention.

 Keywords: Cutaneous leishmaniasis, demographic data,
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 Nahavand
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Poster

The study on different phases of cultivated *Leishmania major* by using glycine max and canavalia ensiformis lectin

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**Background**: Leishmaniasis is one of the important diseases of tropical regions. Considering the importance of obtaining an accurate method to determine the different phases of the parasite in the culture medium and the role of lectins in this context, canavalia and glycin lectins were used to detect the phases.

**Methods:** In the present study, the standard samples of *Leishmania major* were used. To culture the promastigote stages of the parasite, the samples were transferred to RPMI medium with 10% fetal calf serum and 1% penicillin-streptomycin. The culture medium was kept in an incubator at 25 °C. In order to investigate agglutination, concentrations of 10, 30, 60, 120, 240 and 500 µg/mL of glycine and canavalia lectins. The lectins were prepared and placed in contact with promastigotes of the parasite at different stages of growth, then by using a light microscope, agglutination checked out.

**Results**: The present study showed that promastigotes grow rapidly in the logarithmic and less in the stationary phase. This study also examined the effect of agglutination in different concentrations of glycine max and canavalia ensiformis on different stages of *L. major* growth. It concluded that glycine max lectin has greater agglutination power compared to canavalia ensiformis in different stages of parasite growth. Also, the amount of agglutinin was different in various stages and different concentrations. In using glycine concentration of 120 and 240 and in canavalia 120 µg/mL give agglutination in the logarithmic phase, but not in the stationary Phase.

**Conclusion**: Considering that, the percentage of agglutination of each lectin was different in the growth stages of *L. major*, this method could be used to identify its promastigote logarithmic and stationary phases. This capability and technology are of great importance in drug trials and vaccine development.

Keywords: Leishmania major, glycine, canavalia, lectin

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Sth International & 12th National Congress of Parasitology and Parasitic Diseases of Iran Alborz University of Medical, Sciences, Karaj, Iran May 21 - 23, 2024

#### Poster

The trace of yeasts and bacteria in free-living amoeba isolated from COVID-19 patients: concern for secondary infections and transmission of antibiotic resistant strains by endosymbionts

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**Background**: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is responsible for the worldwide pandemic of coronavirus disease 2019 (COVID-19). Free-living amoeba (FLA) are cosmopolitan amphizoic protozoa, which have been reported from different clinical samples and organs. FLAs are believed to carry a couple of microorganisms (fungi, bacteria, and viruses) as endosymbiont.

**Methods:** Nasopharyngeal swabs (n = 60) were obtained from confirmed COVID-19 patients, and cultivated onto the 1.5% nonnutrient agar (NNA) plates. FLA was detected using morphological features and microscopic method under a light microscope. Upon purification of FLA using consecutive cultivation, RNA and DNA extractions were performed to investigate the presence of SARS-CoV-2, yeasts, and bacteria using qReal-time PCR. The quality of RNA was checked by investigating Acanthamoeba spp., housekeeping gene, 18S ribosomal RNA. The genus, species, and genotypes of FLA were characterized using sequencing.

**Results:** From 60 Coivid-19 positive samples, 18 (30%) were positive for FLA. PCR/sequencing results showed the presence of Acanthamoeba sp., Naegleria australiensis, Tetramitus sp., and Vermamoeba vermiformis were in 12 (60%), 1 (4.54%), 2 (9.1%), and 7 (31.81%) of PCR-positive samples, respectively. There was no statistical correlation between the presence of FLA and hospitalization in intensive care unit (ICU), vaccination history for COVID-19, and background diseases. Seven isolates belonged to both T4 and T2 genotypes and five isolates belonged to both T4 and T2 genotypes were showed thermos- and osmotolerans, respectively.

**Conclusion**: Our study is the first reporting the presence of FLA in clinical samples isolated from COVID-19 patients. Although trace of RS-CoV-2 RNA was not detected in isolated FLA, the presence of yeasts, particularly *C. albicans*, and bacteria signifies the probable role FLA.

Keywords: COVID-19, FLA, endosymbionts Correspondence Email(s): maryamniyati@yahoo.com Poster

Third-degree burn wounds healing comparison of using whole-body extraction and secretions of *Lucilia sericata* larvae in vivo

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**Background**: To debridement burned tissues, the wounds generally require physical and chemical debridement to reach the healthy part of the tissue. Maggot therapy is a non-invasive treatment, especially for necrotic and chronic wounds. *Lucilia sericata* fly larvae treat necrotic tissue physically and chemically.

**Methods:** This study aimed to assess third-degree burn wound healing in vivo. This is a controlled experimental laboratory study on the Wistar rats. At first, secretions and extraction of *L. sericata* larvae from a laboratory colony were prepared. Digital photographs of the lesions were tacked on days 7, 14, and 21 after the burn; for histological examination.

**Results**: Both larvae extract and whole-body extracts have beneficial effects in three-degree burn wound healing. The mean level of wound contraction was significantly different between the three treatment groups on days 14 and 21. Also, the mean level of wound contraction in the treatment group with maggot extract and secretions was significantly higher than in the control group.

**Conclusion**: According to findings, the maggot extracts and secretions have sufficient chemical debridement effects to heal third-degree burn wounds in animal models. Since burn wounds are common, it is recommended that further studies be done on the use of maggot therapy on burn wounds in human cases.

Keywords: Wound healing, burn, Lucilia sericata

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Time series model for forecasting the prevalence of some important parasitic infections in slaughtered sheep in northcentral Iran

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**Background**: Uncontrolled parasitic infections in livestock can increase the potential risk of transmission between human societies. Owing to the socioeconomic challenges of these diseases in slaughtered sheep, the current study aimed to forecast the prevalence of these infections in a central slaughterhouse in Alborz province, north-central Iran.

**Methods:** Data from 2009 to 2018 on parasitic infections in slaughtered sheep presented at a slaughterhouse were compiled and the prevalence of these diseases was computed. The prevalence has been considered as a time series and afterward, RStudio software using the best-fitted ARIMA model was applied to forecast the monthly variation in prevalence rates. From 2009 to 2018, a total of 1,339,196 sheep were slaughtered in the studied slaughterhouse.

**Results**: The Iranian Afshari breed was the most slaughtered sheep and, a total of 77.6% of these animals were raised under traditional farming system. In addition to the Alborz province, slaughtered sheep were brought from five other provinces, including Zanjan, Qazvin, Qom, Kurdistan, and East Azerbaijan. The highest and lowest total prevalence of studied parasitic zoonoses in slaughtered livestock were cystic echinococcosis (12.76%) and *Taenia ovis* infection (0.01%), respectively. An approximate stationary trend for fascioliasis and CE and a mild decreasing trend for dicrocoeliasis and sarcocystosis have been forecasted for the next 10 years.

**Conclusion**: The current study has demonstrated for the first time the predicting of some important parasitic infections in sheep in Iran. The results provide helpful data for authorities for controlling these diseases in the frontline of meat production.

Keywords: Predicting, ARIMA model, abattoir, parasitic

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Poster

Time-dependent effects of extracellular vesicles derived from hydatid cyst fluid on the expression of microRNA involved in liver fibrosis

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**Background**: Hydatidosis is a zoonotic neglected disease (ZND) caused by the larval stage of *Echinococcus granulosus*. Evidence suggests a communication between hydatid cyst (HC) and hosts via extracellular vesicles. Extracellular vesicles are produced by protoscoleces, may permeate across LL and fibrotic layer, and manipulate host immune responses.

**Methods:** In the current study, EVs were isolated using ultracentrifugation from sheep HCF and characterized by western blot, electron microscope and size distribution analysis. The effects of EVs on the expression levels of microRNAs (mir-16, mir-29a, and mir-155) involved in liver fibrosis were investigated using quantitative real-time PCR (RT-PCR), 3 and 24 h after incubation.

**Results**: Western blot analyses confirmed the expression of CD63 marker, while Calnexin and CD81 were absent in EVs samples. The SEM and morphology revealed round shape vesicles. The DLS analysis showed average size distribution 130.6 nm diameter. HC-positive human serum showed an interaction with protein lysate of protoscoleces and EVs. The results showed an internalization rate of EVs into THP-1 cells over 60% and bright fluorescent signals at 24 h after incubation. The expression levels of mir-16, mir-29a significantly upregulated after 3 h for 8.66 and 3.420, respectively, while they were significantly downregulated after 24 h for 3.853 and 1.859, respectively.

**Conclusion**: As results, both mir-16 and mir-29a play antifibrotic functions; however, time-dependent effects of EVs in our study suggest that HC may modulate hepatic fibrosis via EVs. Our results demonstrated that EVs might change miRNA expression, which are involved in liver fibrosis.

Keywords: Echinococcus granulosus, EVs, THP-1, MiRNA

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Poster

Anaplasma phagocytophilum in equids, the first survey in Iran

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**Background**: Equine Granulocytic Anaplasmosis (EGA), a common, seasonal, non-contagious, tick-transmitted disease of equids observed where the Ixodes tick vector is founded. Horses less than four years of age have milder clinical signs and may present with only fever and mild depression in Iran.

**Methods:** This study aimed to screen Equidae some regiones of Iran, for EGA. A total of 152 Equidae blood samples (equestrian horses = 100, farm horses = 18, and donkeys = 34) from Mazandaran (Amol and Mahmoodabad Hamedan , Amol, Hamadan (Malair and Hamadan), Arak (Komijan) and Lorestan (Borujerd and Rumshkan) were sampled from April 1401 to June 1402. Blood samples (up to 5 mL) were collected from the jugular vein into the labeled vacuum tubes containing EDTA for PCR analysis and kept at -20 °C until testing. Giemsa-stained thin blood smears were made immediately after peripheral blood collection and further

**Results**: No positive case was found in the microscopic examination while one case (0.65%) was reported positive by the nested-PCR method. Further studies should investigate the *A. phagocytophilum* occurring in other regions.

**Conclusion**: Further studies should investigate the *A*. *phagocytophilum* occurring in other regions.

**Keywords**: *Anaplasma phagocytophilum*, equidae, Iran

**Correspondence Email(s):** z.sadeghidehkordi@basu.ac.ir/dehkordisz@gmail.co m *Toxocara canis* infection in dogs and foxes in Zanjan, Iran

Nastaran Alsadat Tabatabaei Kia <sup>1</sup> Ø, Ali Haniloo <sup>2</sup>, Mehdi Karamian <sup>3</sup>, Negin Torabi <sup>2</sup> ©

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**Background**: A zoonotic helminth, *Toxocara canis*, commonly infects canids and can cause accidental infection in humans. The increase in stray dog population, and the expansion of urban gardens increase the risk of human infection. In this study, the prevalence of *T. cnais* infection was estimated in dogs and foxes.

**Methods:** A total of 484 fecal samples of stray dogs (n = 355), rescue dogs (n = 49), guard dogs (n = 50), and foxes (n = 30) in Zanjan were randomly collected from June 2021 to February 2022. The microscopic examination was done following formalin-ethyl acetate sedimentation procedures. Finally, the PCR method was used to confirm the presence of *T. canis* in positive samples.

**Results**: Microscopic analysis showed that, among 484 samples, 21 (4.3%) tested positive for *Toxocara/Toxascaris* eggs. Only 6 samples from dog feces were identified as a *T. canis* infection through PCR. No fox samples exhibited this infection.

**Conclusion**: The study revealed a slight rise in the prevalence of *T. canis* infection among stray dogs in Zanjan. The presence of dogs in parks and residential areas, increasing the risk of human infection, emphasizes the importance of adhering to treatment and prevention protocols in dealing with stray dogs.

Keywords: Dog, fox, PCR, Toxocara canis

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Toxocara infection and HIV-infected patients with underlying diseases

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**Background:** HIV/AIDS patients with underlying diseases are more susceptible to *Toxocara* infection than other HIV/AIDS patients. The present study describes the results of serological and molecular diagnostic tests applied to a series of suspected cases of toxocariasis in HIV/AIDS patients referred to Shahid Sabze Parvar Behavioral Diseases Counseling Center, ABZUMS.

**Methods:** Blood samples were collected from 105 people living with HIV. Epidemiological data of participant were obtained through a structured questionnaire to investigate the risk factors. Patients CD4+ T cell count were recorded. Anti-*Toxocara* IgG antibodies were detected by ELISA, with a cut-off point of 11. PCR was performed to detect genetic material of *Toxocara* species in the serum samples.

**Results**: Of a total of 105 blood sample collected from HIV/AIDS patients with underlying disease, 12 patients were selected for inclusion in the study. Diagnosis was confirmed by the examinations of hematological, serological and polymerase chain reaction test. In our study, eight cases of all *Toxocara* seropositive people had underlying disease. Additionally, out of the three positive cases identified through PCR testing, two cases had underlying disease.

**Conclusion**: The findings of this study, which was first performed in Alborz province showed that HIV/AIDS patients with underlying disease were significantly associated with *Toxocara* infection, actually underlying diseases makes these patients more vulnerable to the infection. also, relatively high seroprevalence of *Toxocara* in HIV/AIDS people needs comprehensive health education.

Keywords: *Toxocara* infection, underlying diseases, molecular Correspondence Email(s): zibaeim@sums.ac.ir/zahra1994hatami@gmail.com Poster

Toxocariasis and diabetes

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**Background**: Diabetes mellitus type 1 is a common and chronic disease whose prevalence is increasing, and due to its numerous secondary complications, it holds great significance. The aim of this study is to investigate the correlation between the levels of anti-*Toxocara* antibodies and diabetes.

**Methods:** Sera samples from 120 diabetic patients, and 94 non-diabetic controls attending Imam Ali University Hospital, Alborz Province were collected and examined by ELISA for anti-*Toxocara* antibodies. A questionnaire administered by direct interviews was used to collect demographic information and data on associated risk factors.

**Results**: The results indicated a higher prevalence of individuals positive for *T. canis* antibodies in non-diabetic children compared to diabetic children (8.63% vs 3.97%). However, the results with the Fisher exact test was found a significant correlation between the level of *Toxocara* antibodies and incidence of diabetes (P < 0.05).

**Conclusion**: There was significant correlation between the level of *T.canis* antibodies and the incidence of diabetes. Therefore, diabetes can be considered as a stimulus in regulating and modulating the immune system in toxocariasis.

Keywords: Diabetes, Toxocara infection, ELISA

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*Toxoplasma gondii* and *Toxocara* spp. contamination in university area

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**Background**: *Toxoplasma gondii* and *Toxocara* spp. are zoonotic infections may cause severe systemic and ocular illness in infected people. Cats play a major role in environmental contamination and parasite transmission. The goal of the present study was to investigate the prevalence of *T. gondii* and *Toxocara* spp. infection.

**Methods:** Current descriptive study was started by collection of 170 fresh cat fecal samples from deferent sites of Ahvaz Jundishapur University of Medical Sciences area. Sheather's sugar floatation method was applied for all specimens, parasites were identified and microscopically examined. Next, a nested-PCR assay, sequencing and Real-time PCR with high resolution melting curve (HRM) analysis were done.

**Results**: In this study, out of 170 cat fecal samples microscopically evaluated, 8 (4.70%) and 37 (21.76%) were infected with *T. gondii* oocyst and *Toxocara* ova, respectively. Using nested-PCR, 8 samples (4.70%) were shown to be infected with *T. gondii*. HRM analysis showed that all isolates could be sorted into 3 genetic lineages.

**Conclusion**: Considerable prevalence, higher than 50% for *Toxocara* and higher than 25% for *Toxoplasma* in some cases and genetic diversity was obtained in the present study. Hence, it is suggested that all individuals, including children of kindergarten, students, employees, workers, pregnant women that are in contact with the surrounding.

Keywords: Toxoplasma, Toxocara, fecal samples, stray

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Poster

*Toxoplasma gondii* infection affects the complete blood count and disturbs the markers of oxidative stress from the vital organs of wild rodents

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**Background**: Rodents are the synanthropic mammals existing in close proximity to humans and their belongings and have the potential to act as the reservoir for a variety of parasites having zoonotic potential.

**Methods:** Present study was designed to report the molecular prevalence and phylogenetic evaluation of *Toxoplasma gondii* in the blood samples of four wild rodent species [*Rattus rattus* (n = 122), *Mus musculus* (n = 64), *Rattus norvegicus* (n = 57) and *Dryomys nitedula* (n = 1)] that were trapped during May 2022 till July 2023 from three districts in Punjab (Jampur, Dera Ghazi Khan and Multan) and three districts (Upper Dir, Mardan and Bunar) in Pakistan.

**Results**: Results revealed that 44/244 (18%) rodents amplified ITS-1 gene of *T. gondii* through PCR. Parasite prevalence varied between the rodent species. Highest rate of infection was found in *Rattus norvegicus* followed by *Rattus rattus* and *Mus musculus*. For both rat species, *T. gondii* infection significantly varies between the sampling districts. DNA sequencing and BLAST analysis confirmed the presence of *T. gondii* in rodent blood samples. Phylogenetic analysis showed that Pakistani isolates were genetically diverse and clustered with the isolates that were reported from worldwide countries. Complete blood count analysis revealed that parasite infected rodents had disturbed lymphocyte, mean platelet volume.

**Conclusion**: We are reporting a relatively high prevalence of *T. gondii* in Pakistani rodents. Infection leads to disturbed complete blood count and markers of oxidative stress in the vital organs. We recommend large scale studies in various geo-climatic regions of Pakistan to report the incidence and prevalence of this.

**Keywords**: *Toxoplasma gondii*, molecular, prevalence, phylogeny

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*Toxoplasma* infection in patients with myocardial infarction

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**Background**: *Toxoplasma gondii* is a widespread protozoan parasite that infects one third of the global human population. Very little information is known about the impact of *T. gondii* on patients with heart disease. The aim of the present study was to determine the association between *T. gondii* exposure and patients suffering from myocardial infection.

**Methods:** The infection rate of anti-*Toxoplasma* IgG antibodies in 86 patients with myocardial infarction (troponin-T positive) and 86 age and gender-matched controls (troponin-T negative) was examined using enzyme-linked immunoassays. The DNA extraction was performed on separated buffy coats of serologically positive blood samples (32 samples with high titer of anti-*Toxoplasma* IgG). The GRA6 gene of *T. gondii* was amplified using PCR. The existence of polymorphic restriction sites for endonuclease MseI was used with the PCR-RFLP method and the bases of GRA6 gene were sequenced to determine the type of strains (I, II and III).

**Results**: A positive anti-*Toxoplasma* IgG level was found in 61.6% of the myocardial infarction samples and in 24.4% of the healthy controls (P- value 0.05). The PCR results showed that only 3 of the anti-*Toxoplasma* IgG positive patients were found to be positive with GRA6 gene for *T. gondii*. The PCR-RFLP results showed that 2 of the 3 positive sample had 75bp and 623 bp DNA fragments belonging to type II genotype. The sequencing result confirmed the genotype II of *T. gondii*. *Toxoplasma* infection should be considered in myocardial infarction cases.

**Conclusion**: The sequencing result confirmed the genotype II of *T. gondii. Toxoplasma* infection should be considered in myocardial infarction cases.

Keywords: Toxoplasma gondii, myocardial infarction

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Poster

Utilization of bioinformatics methods to develop a new DNA vaccine composed of multiple epitopes to combat the *Leishmania major* 

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**Background**: Leishmaniosis, the most important health problems of the tropical region, is spreading due to the complexity of the treatment and the lack of an effective vaccine. Designing an efficient vaccine for *Leishmania major* is critical. In this study, we designed a multi-epitope DNA vaccine against *L. major*.

**Methods:** We selected five *L. major* antigens (Gp63, LACK, TSA, LmSTI1, and KMP11) to design the vaccine, utilizing information from the Gen Bank database. They predicted epitopes for MHC-I antigens using the Immune Epitope Database (IEDB) and constructed the vaccine with linkers. The designed vaccine, containing 459 nucleic acids, was inserted into the pCDNA3.1 mammalian expression vector. The vaccine's physicochemical properties were analyzed, indicating that it was thermostable and hydrophilic, making it a suitable candidate for further study in animal and human phases.

**Results**: The results section reports the selection of 12 epitopes with high binding affinity and the construction of the Leish21 multi-epitope DNA vaccine. The antigenicity of the vaccine was confirmed using VaxiJen and ANTIGENpro servers. The secondary structure was predicted to consist of alpha-helix, betastrand, and random-coil. The vaccine's physicochemical parameters indicated it was thermostable and hydrophilic. The tertiary structure was modeled using the QUARK server, and the quality of the model was validated through various analyses.

**Conclusion**: They argue that selecting epitopes from protein antigens has advantages over full-length proteins, such as avoiding autoimmunity and immunosuppressive properties. The study suggests that the designed vaccine, Leish21, could be an important option in reducing the .burden of *L. major* infection.

Keywords: DNA vaccine, Leishmania major, bioinformatics

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Visual and spectrophotometric nanobiosensor for Leishmania infantum detection based on kinetoplast DNA probe

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**Background**: *Leishmania infantum* is the causative agent of almost all the human visceral leishmaniasis in Iran. Efforts are being proposed towards the development of cost-effective, noninvasive, sensitive, and specific VL sensing techniques. A DNA biosensor based on gold nanoparticles has been fabricated for the detection of *L. infantum* genome.

**Methods:** The total DNA specimens of seven human and seven dogs buffy coat samples positive for *L. infantum* as well as the total DNA of positive and negative isolates were prepared. A thiolated 24-base oligonucleotide probe from kDNA was functionalized with AuNPs (AuNP-probe). AuNP-probe was then exposed to target and non-target DNA for the hybridization. Dispersion or aggregation of the gold nanoparticles-probe conjugates in the presence or absence of a complementary DNA sequence resulted in an obvious and sensitive change in the UV-vis spectra and the solution color.

**Results**: A red color for the samples containing complementary DNA was observed, whereas, in the samples without complementary DNA, the AuNP-probe turned blue-purple.

**Conclusion**: The results indicated that this method is an easy, reliable, direct, rapid, and cost-effective method for visual detection of *L. infantum*.

**Keywords**: DNA-biosensor, gold nanoparticles, *Leishmania infantum* 

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Poster

Yeast-protozoa interaction in IBS patients: preliminary evidence for synergistic association between *Blastocystis* and *Candida albicans* 

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**Background**: Irritable bowel syndrome (IBS) is gastrointestinal disorder, which could be triggered by the gut microbiome dysbiosis. Molecular epidemiology studies have suggested high prevalence of *Blastocystis* in IBS patients. This study aimed to depict the interaction between *Blastocystis* and selected yeasts in IBS patients.

**Methods:** 52 participants consisted of 25 healthy individuals and 27 patients with IBS, who were categorized based on the presence of *Blastocystis*, were included in our study. Total DNA was extracted and the presence and quantity of some yeasts including *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, *Geotrichum candidum*, *Rhodotorula* spp., *Cryptococcus neoformans*, and *Saccharomyces cerevisiae* were evaluated. *S. cerevisiae* and *C. albicans* were the most prevalent yeasts in both IBS and healthy controls. *C. tropicalis*, *C. parapsilosis*, and *C. neoformans* were not identified in samples.

**Results**: Except G. candidum, the prevalence of all tested yeasts in *Blastocystis*-negative IBS patients were lower than *Blastocystis*-positive IBS patients. The highest quantity of yeasts was observed in *S. cerevisiae* (93357.76 + 244210.86) and *C. albicans* (1041 + 5040.11). Although it was not statistically significant, the prevalence of *S. cerevisiae* and *C. albicans* in *Blastocystis*-positive subjects was higher than *Blastocystis*-negative subjects. The number of *S. cerevisiae* was increased in *Blastocystis*-negative subjects, while *C. albicans* was increased in *Blastocystis*-positive subjects.

**Conclusion**: Regardless the presence of IBS, our findings showed a co-variation between the quantity of *C. albicans* and the presence of *Blastocystis* sp., while the correlation between the number of *S. cerevisiae* and the presence of *Blastocystis* sp., was reversed.

Keywords: Irritable-bowel-syndrome, mycobiome, *Blastocystis*, *C. albicans*, *S. cerevisiae* Correspondence Email(s): hamed\_mirjalali@hotmail.com/hamedmirjalali@sbmu.ac.ir 5th International & 12th National Congress of Parasitology and Parasitic Diseases of Iran Alborz University of Medical, Sciences, Karaj, Iran

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#### Poster

Isolation and molecular identification of *Enterocytozoon* bieneusi and Blastocystis sp. in wild boars in Lorestan, western Iran

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**Background**: *Enterocytozoon bieneusi*, an important Microsporidium, causes chronic diarrhoea in humans and animals worldwide. The intestinal parasite *Blastocystis* sp. is a widely distributed microorganism among humans and various animal hosts. However, there are limited reports about the prevalence of these organisms in wild boars in Iran.

**Methods:** In this study, we collected 52 faecal samples from areas in Lorestan province. After DNA extraction, specific regions (SSU rRNA. ITS) of DNA from *Blastocysts* and *E. bieneusi* were amplified using PCR with primers designed to target conserved regions of their genomes. PCR products were sequenced to determine the genetic sequences of *Blastocystis* and *E. bieneusi* in wild boars.

**Results**: The proportion of PCR-positive samples for *E. bieneusi* was 40.3%, and for *Blastocystis* sp. was 19.2%. Sequence analysis of SSU rRNA and ITS revealed all *E. bieneusi* genotypes as BEB6 and *Blastocystis* sp. subtypes containing ST5.

**Conclusion**: The findings of this study show that wild boars may act as reservoir hosts and carriers of *E. bieneusi* and *Blastocystis* sp, posing a potential risk to human and animal health in the area. This study emphasizes the importance of monitoring and controlling zoonotic diseases in wildlife populations to prevent their transmission to humans and domestic animals.

Keywords: Enterocytozoon bieneusi, Blastocystis sp, wild

boars, western Iran

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Poster

Polyurethane wound dressing incorporating silver meglumine antimoniate chitosan nanocomposite: therapeutic efficacy on cutaneous leishmaniasis caused by *Leishmania major* in BALB/c Mice

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**Background**: In recent years, the antileishmanial activity of nanoparticles has been of great interest. This research aimed to prepare a new polyurethane wound dressing incorporating silver-meglumine antimoniate-chitosan nanocomposite (Ag.MA.CS/PUF) and its evaluation for therapeutic efficacy against cutaneous leishmaniasis caused by *L. major* in BALB/c mice.

**Methods:** This interventional study was conducted in 2022. Polyurethane nanocomposite wound dressing was prepared and incorporated with meglumine antimoniate, silver, and chitosan. This newly synthesized nanocomposite was assessed using FTIR, ICP, XRD, and FESEM.EDS, and TEM. MTT assay was used to measure the toxicity of the prepared synthesized compounds. To confirm the in vivo results, Ag.MA.CS/PUF nanocomposite was administered topically on skin lesions caused by *L. major* (MRHO/IR/75/ER) against 56 inbred BALB/c mice as interventional (n = 42) or control groups (n = 14) once daily for four weeks. Lesion sizes and amastigote counts were measured before, and four weeks after treatment.

**Results**: Synthesis of Ag.MA.CS/PUF was done successfully characterized by analyzing FESEM, EDX, FTIR, XRD, and TEM techniques, yielding significant insights into its properties and structure. Results revealed that the average size of lesions significantly decreased in Ag.MA.CS/PUF group from  $3.02 \pm 0.98$  to  $2.17 \pm 0.33$ mm<sup>2</sup> before and one month after treatment (P < 0.05), but the average size of skin lesions significantly increased from  $3.58 \pm 2.05$  to  $8.73 \pm 5.15$  in the negative control group before and one month after treatment (P < 0.05).

**Conclusion:** Ag.MA.CS/PUF nanocomposite showed a relative therapeutic effect on CL caused by *L. major.* (MRHO/IR/75/ER), it showed a 28% reduction in lesion sizes and an 80% reduction in amastigote counts in the skin lesions four weeks after treatment. Further research with larger sample sizes and blind experiments is recommended.

**Keywords**: Polyurethane wound dressing, *L. major*, BALB/c mice, nanocomposite, silver **Correspondence Email(s):** 

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## Anti-leishmanial activity of ozone on BALB/c mice infected with *Leishmania major*

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**Background**: Cutaneous leishmaniasis is one of the most important health problems in Iran. Considering the disadvantages of glucantim as the main drug for this disease, such as drug resistance, disease recurrence, the remaining scars and drug toxicity, researches on the disease treatment continue to discover new drug compounds. The therapeutic effect of ozone has been observed in recent years. This study investigated the anti-leishmanial effect of ozone.

**Methods:** *Leishmania major* promastigotes were exposed to different concentrations of ozone (5, 10, 20, 30 and 40 ppm) for 5, 10, 20, 30 and 40 minutes and fatality percent was calculated. Next, BALB/c mice were infected with *L. major* and treated with two concentrations of ozone gel (50% and 100%) in each group containing 10 mice. The diameter of the wounds was measured every 7 days during the treatment (4 weeks).

**Results**: The highest fatality percent (100%) of promastigotes was obtained 30 minutes after exposure to 40 ppm ozone gas and the lowest fatality percent was 16.14% after exposure to 5 ppm ozone gas flow after 5 minutes. At the highest concentration of the ozone gel (100%), the wound diameter after 4 weeks of treatment was 9.2 cm compared to 15.3 cm in the control group without treatment.

**Conclusion**: In general, the obtained results show that ozone alone cannot completely heal the wound, but can be used as a complementary treatment with the main drugs.

**Keywords**: *Leishmania major*, ozone, BALB/c mice, fatality percent

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#### Poster

The role of circular RNAs in the diagnosis and molecular mechanism of *Leishmania infantum* and *Leishmania tropica* 

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**Background**: Leishmaniasis is a group of vector-borne diseases caused by protozoan parasites of the genus *Leishmania*, leading to a range of clinical manifestations from self-healing skin ulcers to potentially fatal visceral infections. Circular RNAs (circRNAs) are a class of non-coding RNA molecules that are highly stable due to their closed-loop structure. CircRNAs, which play a role in various biological processes, have emerged as potential biomarkers for various diseases due to their stability, abundance, and dysregulated expression in pathological conditions. In this study, we aimed to investigate the effect of circRNAs in *Leishmania* parasites and their potential as diagnostic markers and therapeutic targets.

**Methods:** The human leukemia monocytic cell line (THP-1) was used in invitro model of *Leishmania* infection. THP-1 cells were previously differentiated into macrophages by phorbol 12 myristate 13 acetate (PMA) treatment. Differentiated THP-1 cells were infected with *L. infantum* and *L. tropica* promastigotes in the stationary phase. The gene expressions of circRNAs were determined 24 hours later by RT-PCR technique. In addition, the gene expressions of mRNAs associated with differentially expressed circRNAs (DEGs) were examined. The molecular pathways associated with DEGc were then analyzed using KEGG platform.

**Results**: This study revealed that 12 circRNAs were differentially expressed in THP1 cells infected with *Leishmania* (6 DEGs for *L. infantum* and 6 DEGs for *L. tropica*). These results suggest that some circRNAs may be potential biomarkers for diagnosis in *Leishmania*-infected patients. KEGG enrichment analysis showed that DEGs were mainly involved in regulation of host cell cycle, apoptosis, cellular immunity, signaling pathway and other processes.

**Conclusion**: This is the first study to provide an overview of circRNAs from *Leishmania* and elucidate the mechanisms of a network of circRNAs and mRNAs in a model of *Leishmania* infection.

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