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Oral Presentations
Tropical theileriosis (Theileria annulata infection) was discovered in North Africa (mainly Algeria) during the early 20th century by Sergent, Donatien, and Lestoquard. They studied this disease (but also anaplasmosis and babesioses) in Algeria, Morocco, and Tunisia. These authors were the first to describe the reproductive stage of *T. annulata* in ticks. They were also the first to develop a live vaccine (Kouba strain) against this parasite, which was used in the Moroccan, Algerian, and Tunisian fields. Tropical theileriosis is transmitted seasonally by *Hyalomma* ticks, in Mauritania, *H. dromedarii* is its vector, whilst, in Morocco, Algeria, and Tunisia, the vector tick is *H. scupense*. Few populations of *H. lusitanicum* were reported in Algeria but no information about its vector role is available. No information is available in Libya about tropical theileriosis and the vector tick. In the Maghreb region, this disease is occurring under two states: (i) enzootic stability where the seroprevalence reaches 100% and (ii) enzootic instability, occurring mainly in exotic pure-breed cattle farms. The first is most frequent in Northern parts of the Maghreb region where the tick burdens are high. A cell culture vaccine was developed in Morocco and Tunisia but these vaccines were never used in the field and the control of this disease is limited to treatment of clinical cases and acaricide application. The number of scholarly published papers is very low in Maghreb region; this is in contradiction with the importance of this disease. Tropical theileriosis seems to remain enzootic in Maghreb since the remote regions and the presence of poorly managed farms are still frequent. The research activity seems to be weak (and even totally absent) in most of the Maghreb countries. The number of ongoing projects is low, that's why, international collaborative programs are needed to improve our knowledge on this disease. A Maghreb network is needed urgently to harmonize the research, the extension and the control programs.
MicroRNAs (miRNAs) are key regulators of many biological processes including development, cell proliferation, metabolism, and signal transduction. Schistosomes are parasitic worms that cause the neglected tropical disease schistosomiasis. Adult female schistosomes produce large numbers of eggs that are primarily responsible for the disease pathology and critical for dissemination. Consequently, understanding schistosome development and egg production are important in both the pathology and in disease transmission. We systematically identified *Schistosoma japonicum* (*S. japonicum*) miRNAs in the key stages of male-female pairing, gametogenesis, and egg production using small RNA deep sequencing, resulting in the identification of 38 highly confident miRNAs, including 10 previously unknown miRNAs, many of these miRNAs are differentially expressed between male and female schistosomes and during different stages of development. Then, we identified 29 target genes for 15 of the *S. japonicum* miRNAs and found that suppression of several female enriched miRNAs (bantam and miR-31) led to morphological alternation of ovaries and aberrant expression of eggshell proteins in female schistosomes. In addition, we found extracellular vesicles secreted from *Schistosoma japonicum* could transfer their cargo miRNAs to recipient cells and then regulate the expressions of target. These findings uncover key roles for miRNAs in schistosome sexual maturation and pathogen-host interaction that may facilitate the development of new interventions for the control of schistosomiasis.
Cytokines are the key factors in innate as well as in acquired immunity. These soluble mediators act on various cells irrespective of type of antigens and manipulate cell physiology to counter the attack of a foreign body. A variety of cytokines are produced and activated by the interaction of antigen with immune cells and act together forming a cytokinome. Their secretory equilibrium is essential for endowing resistance to the host against toxoplasmosis. A narrow balance between Th1 and Th2, but also of Th17 cytokines (as demonstrated until now) is essentially required for the development of resistance against *T. gondii* as well as for the survival of host. Recent clinical studies have showed that virulent strains determined Th2 deviated response and are at the basis of severe South American ocular toxoplasmosis, suggesting that immunomodulatory therapeutic approaches can benefit patients with this important cause of visual damage for humans.

Keywords: *Toxoplasma gondii*, toxoplasmosis, cytokine, interferon, interleukin
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TITLE

Characterization of Tick-Host-Pathogen Interactions for Developing Vaccines Against Tick-Borne Diseases

Ticks are blood feeding arthropod ectoparasites that transmit pathogens causing diseases in humans and animals worldwide. New approaches for tick control are dependent on defining molecular interactions between hosts, ticks and pathogens to allow for discovery of key molecules that could be tested in vaccines for control of tick-borne diseases. The complex molecular interactions between ticks and pathogens are analyzed by a systems biology approach that allow the integrated analysis of metabolomics, transcriptomics, proteomics and other omics datasets for discovery of key pathways and molecules that mediate tick-pathogen interactions. A vaccinomics approach could then be used to identify and fully characterize candidate protective antigens and validate vaccine formulations, including development of effective screening platforms and algorithms for discovery and validation of candidate protective antigens. Tick vaccines combining tick-derived and pathogen-derived antigens that affect both tick infestations and pathogen infection and transmission could be developed for the control of vector-borne diseases.
Host Parasite Coevolution between *Toxoplasma gondii* and the House Mouse

Toxoplasma gondii is a protozoan parasite distantly related to the malarial genus, *Plasmodium*. It has a complex life cycle, in which true cats (Felidae) and above all the domestic cat plays the role of definitive host in which meiosis, gametogenesis and gametic fusion occurs. Products of meiosis are excreted into the environment as many tens of thousands of environmentally resistant oocysts in feces. Oocysts differentiate by mitosis into sporocysts which may be eaten inadvertently by foraging animals many months later. It seems as if any warm-blooded animal can support the hatching and differentiation of sporozoites into the fast replicating tachyzoite form. These infect the intermediate host and initiate several rounds of replication in intracellular parasitophorous vacuoles. If the immune response of the intermediate host is inadequate the parasite continues to replicate, exciting a cytokine storm and killing the host within less than 10 days. However most mammalian hosts control the replication phase and stimulate the parasite to enter the slowly replicating bradyzoite stage characterized by the formation of cysts in brain and muscle. In this condition the parasites persist for the life of the host, waiting for the host to be caught and eaten by the next cat.

Since nearly all definitive hosts of *T. gondii* globally are domestic cats, whether pets or feral, the typical intermediate hosts of the parasite are cat prey animals, small birds including pigeons, and mice, that live in close proximity to humans. Our work has established the basis of *Toxoplasma* immunity in domestic mice. We have shown that there is in mice a specific resistance mechanism based on a dedicated family of interferon-inducible GTPases, the IRG proteins (Immunity-related GTPases). These interferon-inducible proteins bind to and destroy the parasitophorous vacuole membrane, killing the parasite. However some *T. gondii* genotypes appear to defeat the IRG system and are not controlled. They rapidly overgrow and kill the host. Virulence is due to the secretion of an active kinase complex into the host cell that inactivates the effector IRG proteins. These virulent *T. gondii* strains are in turn resisted by mice from the Indian sub-continent, belonging to the subspecies *Mus musculus castaneus*. Genetic experiments show that these mice carry an additional IRG gene allele that can suppress the virulent *T. gondii* strains. We show how this is done.

Both mice and domestic cats are Old World species, arising about 10,000 years ago in Asia Minor at the dawn of early agriculture and urbanization. *T. gondii*, on the other hand, is a New World species, probably arising many 10s of millions of years ago in the S. American sub continent. In Brazil and Colombia *T. gondii* has diversified into a remarkably large number of strains, most of which are unknown in Eurasia and are highly virulent for *Mus musculus*. However the domestic mouse arrived in S. America about 500 years ago and has persisted there. Thus it is to be expected that *Mus musculus* in South America will demonstrate recent adaptation to the local strains of *T. gondii*. We will present evidence that this is indeed the case, involving selection of a specific resistance allele from the standing variation available among the Eurasian mice that reached Brazil 500 years ago. Our research thus stretches from basic immune processes and their biochemistry to the study of host-parasite co-evolution on a global scale.
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Due to significant importance of Foodborne Parasitic Diseases (FPD), Foodborne Disease Burden Epidemiology Reference Group (WHO) was launched as a 7-yr project to estimate the burden of food borne diseases in terms of DALY, YLD, LLD besides incidence and prevalence. Many papers were published in authentic journals and here is a bird’s eye view on what we reported focusing on Middle East countries. Food Borne trematodes (FBT) results in approximately 665,000 DALYs a year. In Iran, two outbreaks as for fasciolosis in 1989 and 1999 involved 7000 and 10,000 cases, respectively. Iran, Iraq, Pakistan, Saudi Arabia, Kuwait, have fasciolosis more or less. Clonorchis sinensis and Opistorchis viverrini are mainly found in East and South East Asia and the Asia Pacific regions. The number of people infected with liver fluke is 25 million with 10 million for O. viverrini, 15 million for C. sinensis and about 1 million for O. felineus. There have been 15,000 to 20,000 total cases of anisakiasis and 12-100 cases per year for diphyllobothriasis in Asia. The overall annual cost of cystic echinococcosis in Iran was estimated at US$232.3 million (95% CI US$103.1–397.8 million), including both direct and indirect costs. Iraq, Jordan, Pakistan, Afghanistan, Palestine are among other countries of hydatidosis. Other cases are as follows: Fasciolopsiasis 0%-92.9%, trichinellosis 0.9%-9%, cysticercosis in China in 29 provinces with 7 million cases, toxoplasmosis 1.8%-51.8%, opistorchiasis 1.4%-86%, clonorchiais 1%-54.2%, paragonimiasis 0.5%-40%, anisakiasis: 15,000 to 20,000 total cases, giardiasis 1%-10%, amoebiasis 1%-58%, ascariasis: 0.1%-95%, angiostrongyliasis in China including 160 cases in outbreaks, trichuriasis: 0.5%-89%, and hookworm disease 0.1%-85%. Specific food habit among Asia countries has boosted the rate of infection with FBD.

Keywords: Food Borne Diseases, Parasites, Middle East
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**TITLE**

Advance on the Piroplasms in China

The advance on the piroplasms in China in recent three decades was reviewed. The studies of *Theileria* and *Babesia* were conducted in 5 institutions. Sixteen species of *Babesia*, *B. bovis*, *B. bigemina*, *B. major*, *B. ovata*, *B. orientalis*, *B*. sp. Kashi, *B. motasi*, *B*. sp. Xinjiang, *B. caballi*, *B. gibsoni*, *B. divergens*, *B. ovis*, *B. trautmanni*, *B. perronciotii*, *B. hongkongensi*, *B. canis*, and 47 species of *Theileria*, *T. annulata*, *T. orientalis*, *T. sinensis*, *T. ovis*, *T. uilenbergi*, *T. luwenshuni*, *T. equi* have been identified. The phylogenetic relation and genome were analysed. The vector ticks of most of these piroplasms were verified by transmission experiments. *Boophilus microplus*, *Hamaphysalis longicornis* and *H. qinghaiensis* were proved to be the dominant ones. Epidemiological investigation was also performed in some provinces or regions in China. Both serological assay, e.g. ELISA, and molecular tools, e.g. PCR, RLB, LAMP, RPA, were developed for detection of infection of piroplasms. Recombinant proteins as vaccine candidates have been tested in laboratory and partial protection was observed.
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Ticks are hematophagous arthropods, notorious as vectors of human and animal pathogens. Blood feeding by ticks requires prolonged contact with host tissues and blood, and it has been suggested that the co-evolution of ticks with their natural hosts has resulted in selection of an appropriate set of salivary components allowing the tick to evade both specific and nonspecific host immunity in order to successfully obtain its blood meal. In their quest for a blood meal, ticks inject a cocktail of bioactive molecules to their vertebrate hosts. Understanding the molecular interactions between tick vector and vertebrate host might help in developing strategies to combat tick and tick-borne infections. Moreover, characterizing those tick salivary proteins critical for feeding are essential to developing a molecular basis for new vaccines and therapeutics against tick and tick-borne pathogens. Together, tick proteins that facilitate pathogen infection represent potential candidates for vaccine and/or drug development. Our long-term goal is to reduce or block the spreading of tick and tick-borne diseases by interfering with vector proteins. This research project establishes the biological relationship and significance of novel tick salivary proteins as anti-tick vaccine to block tick feeding success and/or survival and replication of pathogen in the vector. During this research project, several activities were carried out in order to overcome the difficulties and to test our central hypothesis that Rhipicephalus microplus salivary immuno-modulatory proteins are novel vector antigens that can be exploited for the development of broad-spectrum anti-tick vaccines to prevent tick feeding and pathogen infection. We accomplished the objective of this proposal by pursuing the specific aim using a combination of molecular, physiological, biochemical, and genetic approaches, a- Bioinformatics analysis to identify and investigate the R. microplus antigenic proteins b- recombinant protein expression c- Cross reactivity analysis of R. microplus recombinant protein with R. sanguineus infested rabbit serum d- Reactivity analysis of R. sanguineus tissues with R. sanguineus infested rabbit serum. This research may provide a proof of concept to investigate the immune-protective potential of expected antigens against R. sanguineus/R. microplus in order to develop a broad-spectrum anti-tick vaccine. Ideally, an anti-tick vaccine would be based in a secreted molecule, exposing the host with each tick bite thereby providing for natural boosting.
The gastrointestinal nematodes are common pathogens in grazing sheep/goats throughout the world which impairs productivity and leads to high economic losses. To access the nematodes prevalence trend, drug resistance/efficacy in small ruminants two ecologies i.e., cold-dry area of Ziarat Balochistan and hot-humid area of Chakwal were selected for experiment. The results showed that the sheep from Ziarat area were lower (29%) prevalent with nematodes compared to Chakwal area (66%) based on faecal sample analysis. Four major nematodes like Nematodirus, Strongyloides, Haemonchus, Trichostrongylus and Trichuris were recorded at Ziarat area. In Chakwal area except Nematodirus all these three nematodes and in addition Oesophagostomum, Chabertia, Trichuris, Ostertagia were also recorded. The younger and older female-animals (one and five years) presented higher nematodes prevalence during autumn. The resistance/efficacy of three available synthetic anthelmintic (Oxfendazole alone, oxfendazole-Levamisole combination and Ivermectin) were assessed against natural major nematodes infected sheep and goats. Overall results revealed susceptibility of these anthelmintics (97-99% Confidence Interval of Faecal Egg Count Reduction-FECR) against four nematodes and no evidence of resistance recorded. However, three herbal anthelmintics (Atreefal Deedan, Deedani and Kirmar) available in Pakistan were tested against nematodes as an option of alternate remedy. Among these Atreefal Deedan showed highest (90-96%) FECR, followed by Deedani (80-83%) and Kirmar (32-60%). It is concluded that, on small scale assessment no drug resistance observed against few worms in sheep and goats in Pakistan. A broader study is recommended for assessment of drug resistance and also evaluation of available or practiced anthelmintic.

Keywords: Nematodes, Anthelmintic Resistance, Herbal Anthelmintic, Sheep, Goats
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**TITLE**

Taeniasis: A Neglected Zoonotic Disease and its Review

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Taeniasis; a worldwide parasitic disease but rare in muslim countries is caused by eating undercooked or raw meat from bovine or pork infected with Taenia saginata, Taenia Solium, Taenia ovis and Taenia asiatica. The disease is mostly mild or symptom less, but sometimes it lead to more serious condition cysticercosis, epilepsy. There are 32 reported species of Taenia but few noted above have significance impact on causation of Taeniasis. All races, genders and ages are equally susceptible to infection, however meat consumption has direct effect. Various vital organs may be involved including abdominal cavity, sub cutaneous tissues, muscles, spinal cord, central nervous system, brain, heart, and eye and involve anorexia, abdominal pain, weight loss, sizures, neurologic signs, meningitis and altered mental status. The mortality rate generally is very low but may be complicated as stroke, hydrocephalus and encephalitis. Anthelmintic therapy: Albendazole, praziquantel, niclosamide and or both in combination is the drug of choice. Surgical intervention is required in ventricular, ocular and spinal lesions along with anthelmintic drugs. Diagnosis id based on stool examination of stool for the presence of ova and proglottids in fecal materials and serological testing, immunoblot assay along with CT scan and/or MRI for patients with CNS symptoms.

**Keywords:** Taenia, clinical signs, diagnosis and therapy
The objective of this study was to estimate economic losses in terms of milk production caused by tick infestation in dairy buffaloes (Bos bubalus bubalis) of Punjab (Pakistan). To this end, six hundred Nili-Ravi buffaloes infested with *Hyalomma* ticks (Acari: Ixodidae) were selected and divided into two equal groups viz; A & B. The animals of group A were treated with various doses of 5% cypermethrine pour-on (Cipermetriven, Ivan Labs, Spain) while those of group B were treated with propylene glycol (Propandiol - (1, 2), Merck) as a sham treatment. Average milk production (L) and butter fat (%) was recorded before and after treatment in order to calculate post-treatment increase in these parameters (if any). An average daily increase of 1.15L in milk yield per animal with 1.31% more fat was observed in acaricide-treated animals. A dose-dependent effect of acaricide was found on the number of ticks as well as milk production and fat. The results provided a baseline data for further research on economic impact of tick nuisance to the smallholder dairy farming systems of Pakistan.

**Keywords:** Hyalomma, Bos bubalus bubalis, Ticks, Pakistan
Cockroaches, an urban pest infested human dwellings act as mechanical vector of various bacteria, fungi and parasitic contaminant. Their biology and physiology favor them as an ideal vector therefore Ova and cysts of parasitic organism may settle into the crevices and cracks of their thorax and head. This study investigated external parasitic contaminants of cockroaches collected from houses and hospitals of Lahore. Direct wet smear, 1% lugols iodine and modified acid-fast bacilli staining were used to identify the parasites from the external body surface of cockroaches. *P. americana* harbored more parasites as compared to *B. germanica* in both environment. *E. histolytica* protozoan was found as the most prevalent followed by *E. vermicularis* however, *A. lumbricoides* were least prevalent in hospitals and houses. Simpson Diversity index value of parasitic contaminants isolated from *B. germanica* collected from houses was 0.92133 and 0.91827 for hospitals that indicated *B. germanica* has lowest diversity of parasitic contaminants. *B. germanica* found in indoor sites of hospitals and had more chances to encounter with filthy habitat and fecal waste in bathrooms. The Shannon-Weiner diversity index calculated value was found highest for *P. americana* at both sites houses and hospitals as 2.554291 and 2.536765 respectively, which predicted that the rate of parasitic contaminants of both species was not even. Both experimental sites were not significantly different in carriage of parasitic contaminants on cockroaches (F (1,6) =1.795, P= 0.229). These variations in parasitic contamination can be explained as both cockroach species has varied ecology, habitat and chance to encounter with parasites while foraging for food. Effective control strategies will reduce the public health burden of the gastro-intestinal parasites in the developing countries.
**First Molecular Characterization of Leishmania species from Waziristan, Pakistan**

**Background:** War, infection, and disease have close relationship. Military operations against terrorist activity in the tribal areas of Khyber Pakhtunkhwa (KP), Pakistan has caused the largest human displacement of local people to urban areas. A neglected consequence of this tragedy has been the recurrent epidemics of cutaneous leishmaniasis. Waziristan is a tribal area on Southern border and is under military operation against terrorism since last 4 years. CL is a major and fast increasing public health problem among the local Pakistani population of this area. The Internally Displaced People (IDPs) are main source of bringing CL to other urban areas of KP. Epidemiological and molecular studies could not be conducted in the past during Taliban regime. During an epidemic outbreak of CL in Waziristan, a large-scale epidemiologic study was carried out from May 2014 to May 2015 to determine the prevalence and molecular diagnosis of the CL in local population. 15230 individuals were surveyed in 25 different villages. A total of 856 CL cases with active CL lesions and scars were identified and 432 skin biopsies were collected from active CL lesions, confirmed by Giemsa stained microscopy and kinetoplast DNA (kDNA) and finally characterized by ribosomal internal transcribed spacer 1 (ITS1) amplification and subsequent restriction fragment length polymorphism (RFLP) analysis using *Haemophilus* III enzyme. The total prevalence of CL in local population was found to be 5.6%, with active lesions and scar prevalence of 3.4% and 1.6% respectively. The microscopic examination was positive for 224/432 (52%) samples, kDNA PCR positive samples were 423/432 (98%) while ITS1-PCR was positive in 357/432 (82.6%) samples. *Leishmania* species characterization by ITS1 PCR-RFLP analysis confirmed *Leishmania tropica* in 345/357 (96.6%) of ITS-PCR positive samples. *L. major* was found in 12/357 of positive cases, all of them with travelling history to Afghanistan. This study has resulted in identification and characterization of *L. tropica* species in Waziristan for the first time and confirmed the previous epidemiological reports from Southern KP province that were based on prevalence studies only. We here highlight the urgent need of a regional / national leishmaniasis (vectors and reservoirs) control program in the military operated area. As IDPs from these areas are continuous source of CL to the urban areas of Khyber Pakhtunkhwa, Pakistan.
Little is known about the occurrence of apicomplexans parasitic DNA in the muscles of rock pigeons (Columbia livia). The muscles from 126 (brain and heart from 123 and brain from only 3) rock pigeons were inspected for DNA of Toxoplasma gondii, Neospora caninum, and Sarcocystis species by using PCR. Seventy were male and 56 were females. The birds were part of flock of pigeons housed at the tombs of Saints in Multan, Pakistan. Dead and poor conditioned euthanized birds were submitted at the Department of Pathobiology, Bahauddin Zakariya University, Multan, Pakistan for necropsy. Brain and heart tissues were isolated and brought to Center for Molecular Medicine and Infectious Diseases, Virginia Tech. USA where DNA extraction and PCR was performed. Forty-nine (37.3%) of the birds were confirmed as positive for DNA of T. gondii positive birds, while the heart was infected in 41 (32.5%) of brain positive birds. Seventeen (13.4%) of the pigeons, 11 male and 6 female, were positive for N. caninum. The N. caninum DNA distribution was highly variable in the muscles of rock pigeons that of T. gondii and was detected in the heart of only 2 (female), heart and brain of 11 (male), and the brain of 4 birds (male). Two of the 126 rock pigeons (female) were positive for both T. gondii (heart and brain) and N. caninum (heart only). Amplicons of Neospora caninum were sequenced and found nucleotide identity of 91–97% with N. caninum. Sarcozystis DNA was not found in any bird. The T. gondii prevalence in rock pigeons and their predation on by dogs and cats recommends that these may play an important role in the epidemiology and maintenance of pathogen in the environment. Our study specifies that rock pigeons are acting as intermediate hosts and this data will help in the understanding of epidemiological features of N. caninum.

**Keywords:** Toxoplasma, Neospora, Sarcocystis species, Rock pigeons, Columbia livia
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Among the parasitic zoonoses Toxoplasmosis has the highest incidence causing serious threats to animals and human population. The present study was conducted to investigate the seroprevalence of Toxoplasmosis in goats and its haematobiochemical evaluations. A total of 384 samples were collected randomly from all the three tehsil of district Charsadda and screened for Toxoplasma gondii infection through Latex agglutination test (LAT). Out of total, 218 samples were seropositive for Toxoplasmosis showing an overall prevalence of 56.77%. The seroprevalence of T. gondii infection was 55.46%, 57.03% and 57.81% in Tehsil Tangi, Charsadda and Shabqaddar, respectively. Toxoplasmosis was more prevalent (66.14%) in female goats as compared to male goats (47.39%).

The prevalence of Toxoplasmosis was significantly (P<0.05) higher in goats of age group ≥ 2 years (70.83%) than goats of age group < 2 years (42.70%). The consequences of haematological parameters such as Hb, WBC, RBC, PCV, Lymphocyte, Monocytes, Eosinophils and Basophils of the infected goats were 5.62±0.300, 2.70±0.203, 4.98±0.338, 4.98±0.338, 14.85±0.417, 40.29±0.726, 2.08±0.147, 5.11±0.158 and 0.22±0.78, respectively. These values were significantly (P<0.05) decreased in comparison with healthy goats. However Neutrophils (69.63±1.727) were increased in infected than non infected goats. Serum biochemical investigations such as ALT, AST, LDH, BUN, and T. Protein of the infected goats were 214.60±2.702, 386.30±1.809, 416.50±3.634, 61.40±1.231 and 15.46±0.266, respectively. Which were significantly (P<0.05) increased in infected than healthy goats. While Albumin level (1.47±0.152) was decreased in infected than non infected goats. It is concluded that the Toxoplasmosis is prevalent in district Charsadda. The significant increase in Neutrophils, liver enzymes and Blood Urea Nitrogen level suggests the acute nature of the disease with hepatotoxic and nephrotoxic consequences.

**Keywords:** Prevalence, *Toxoplasma gondii*, goat, LAT, haematobiochemical evaluations, hepatotoxic, nephrotoxic.
Studies were conducted on biology and economic importance of goat grubs, *Przhevalskiana silenus*, in upland of north-east region of Balochistan, Pakistan, during April 2011 to March 2012 with the objectives to observe different stages of the life cycle and to estimate the economic importance of hypodermodosis in goats for better control strategies. All the observations regarding the adult fly activity season, oviposition, first, second and third instars larval and pupation period were recorded in naturally infested goats in the study areas. Depending on the geo-climatic conditions of the areas under study, there were variations of not more than a few weeks regarding completion of the life cycle in a complete one year period of study. First instars larvae (L1) were observed from mid of May to mid of July, second instars larvae (L2, 12 mm) from mid of July to November, third instars larvae (L3, immature 16.1 mm, mature 18 mm), as well developed warbles on the back and flank region of the goats, were palpated in the field and collected from both the slaughter houses and fields from December until the end of February. The pupal period was observed in early spring in the months from March to mid of April. The adult flies were not seen in the field due to their very short life period. However, the adult fly activity season (Oviposition period) was observed in early summer from mid of April to mid of May. This information was collected from the animal’s owners and shepherds that animals run suddenly (gadding), become restless and do not graze and feed properly. The larvae at different stages of their life cycle were isolated, preserved, processed and identified. The overall losses due to warble fly infestation in the study areas were calculated as Pak. Rupees 7578625.49 (Pak. Rs 7.57 million = US$ 77530.69, US$ 1 = 97.75) annually. It is concluded that the best time for treatment of goat warble fly infestation in northern mountainous region of Balochistan, Pakistan, is in the months of June and July when first larval instars are still in migratory stage and have not yet caused damage to the skin.

**Keywords:** Biology, Economic losses, *Przhevalskiana silenus*, Upland, Balochistan, Pakistan.
Report of *Toxoplasma gondii* in Butcher Community in the Municipality of District Mardan, Khyber Pakhtukhwa, Pakistan

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Toxoplasmosis, a globally distributed zoonotic parasitosis, caused by *Toxoplasma gondii*, with members of the Family Felidae (Felis catus) as definitive hosts and an exceptionally broad range of intermediate hosts including humans, birds, domestic and wild animals. In order to assess, *Toxoplasma gondii* infection in butcher community, three hundred Serum samples (n=300) were screened using latex agglutination test (LAT) to detect IgG specific immune responses. Out of 300 examined serum samples, 90 samples were confirmed antibodies against *T.gondii*. The overall seroprevalence rate of *T.gondii* infection amongst the butcher community of District Mardan was 30%. Based on statistical analysis, relationship between age and *T.gondii* infection revealed significant association (*P*<0.05) with highest prevalence rate (42.9%) in age group >60 and lowest (11.1%) in age group <20. *Toxoplasma gondii* infection rate was significantly high (*P*<0.05) in Tehsil Takht Bhai (40%) followed by Tehsil Katlang (29%) and lowest in Tehsil Mardan (21%). It was concluded from the study that butchers are confronted to latent infection with *T.gondii* along with high prevalence rate in rural area as compared to urban areas. Proper screening strategy maybe essential to make access of the free toxoplasma meat to the community at butchery.

**Keywords:** Human, Latex agglutination test, Seroprevalence, and *Toxoplasma gondii*
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TITLE

Serdignosis of *Toxoplasma gondii*

Prevalence in Male Psychiatric patients of Lahore, Pakistan

Toxoplasmosis is a disease caused by coccidian parasite *Toxoplasma gondii*. Its infection is prevalent all over the world including Pakistan. It mainly affects the warm blooded animals including livestock and humans. When humans consume raw and under cooked meat containing tissue cysts or drink water containing infected oocytes of *T. gondii*, toxoplasmosis occurs. Risk of *T. gondii* infection is higher in psychiatric patients because of their brain malfunctioning and improper behavior increases the risk of transmission. Blood samples from 120 male subjects were collected to examine the prevalence of *T. gondii*. Serum was separated from blood by using centrifuge machine. Then by using commercially available IgG ELISA kit prevalence (%) of *T. gondii* in blood samples was detected. The percentage prevalence of anti-*T. gondii* in overall male subjects was 34.1%. In case and control group percentage prevalence of anti-*T. gondii* was 46.7% and 21.7% respectively. The prevalence (%) of anti-*T. gondii* IgG antibodies in various psychiatric patients was 75 in bipolar disorder, 57.1 in schizophrenic, 57.1 in epilepsy, 50 in anxiety, 50 suicidal, 35.7 in depression and 27.2 in aggressive patients. Result revealed that percentage prevalence of *T. gondii* in psychiatric patients was high as compared to control group. Safety measures are suggested to avoid the infection of toxoplasmosis in psychiatric patients. Present study will help in improving health status of psychiatric patients as well as general public by reducing the transmission risk.
Seroprevalence of *Toxoplasma gondii* and its Correlation with ABO Blood Group Frequency among Females of District Mardan

*Toxoplasma gondii* is an obligate parasite causing zoonotic infection known as toxoplasmosis. *T. gondii* is worldwide in distribution and may cause various clinical problems in warm blooded animals such as birds and mammals. ABO blood group phenotypes are defined by the molecules that consist of carbohydrate, are present in the structures of glycoproteins and glycolipids expressed in red blood cells and other tissues. The present study was designed to determine the seroprevalence of *T. gondii* and its correlation with ABO blood group frequency among females of district Mardan. The study was multidisciplinary in approach using questionnaire survey for assessment of general health status and to find out risk factors. Blood group phenotyping was done by using antiserum A, B and D. Seroprevalence of *T. gondii* was also determined by using ELISA technique. Results showed that B Blood group was more prevalent (34%) among study population as compared to other blood groups. Overall prevalence rate among female was 41.11%. Age wise comparison was also done that showed that female respondents belong to elder age group (43-50 yr) was more seropositive for anti-*T. gondii* IgG antibodies. Analysis of data showed that seroprevalence rate was more in those respondents who were illiterate, married, had low socioeconomic status and poor hygienic conditions. Data analysis showed no significant association between ABO blood group and prevalence of *T. gondii* infection. More studies are recommended on susceptible groups for creating awareness among general population and for better public health. It was strongly suggested that more studies are needed by using molecular techniques i.e. PCR for proper diagnosis of *Toxoplasma* infection.
TITLE

Retrospective study on Lab Samples for Diagnosis of Fecal and Blood Parasite of Small Ruminants in Pakistan

To find the prevalence of parasitic infection in small ruminants in Pakistan. Setting: The study was conducted from September 2016 to August 2017 in University Diagnostic Lab, UVAS, Lahore. A total of 1932 samples (1051 fecal, 881 blood) of sheep and goats housed in various areas of Pakistan were received and processed in University diagnostic lab (UDL) for examination of fecal and blood parasites. Floatation and Sedimentation techniques were applied for fecal while blood parasites were examined under microscope. Out of 1051 fecal samples, 702 (66.79%) were found positive for fecal parasites. *Eimeria* spp 267 (25.4%) was found significantly higher (p<0.05) as compared to *Ostertagia circumcincta* 186 (17.69%), *Trichostrongylus* spp 80 (7.6%), *Haemonchus contortus* 78 (7.42%), *Fasciola hepatica* 51 (4.85%) and *Trichuris globulos* 40 (3.8%). Out of 881 blood samples, 575 (65.26%) samples showed positivity for haemoparasites. *Theileria* spp 532 (60.38%) was found significantly higher (p<0.05) as compared to *Babesia* spp 22 (2.49%) and *Anaplasma* spp 21 (2.38%). In the light of this retrospective study, it is concluded that there is a high prevalence of parasitic infection in small ruminants in Pakistan, so proper control strategies should be implemented to control the parasitic infection.
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<th>Muhammad Arfan Zaman ⁺, Asfand Yar Khan², Muhammad Fiaz Qamar ⁺, Abdual Shakoor²</th>
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<td>1. Section of Parasitology, Department of Pathobiology, College of Veterinary and Animal Sciences Jhang, Sub-Campus University of Veterinary and Animal Sciences, Lahore, Pakistan</td>
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<tr>
<td>2. Department of Clinical sciences, College of Veterinary and Animal sciences, Jhang Pakistan</td>
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This study was an effort to collect first hand information about current status of ecto-and-endoparasites in pigeons in and around Jhang, Pakistan. A total of 71 (n=71) (Domestic breed) infected pigeons, on the base of clinical signs, were purchased from different fanciers of pigeons. Antemortem and postmortem examination were carried out for ecto-and-endoparasites, respectively. Head, neck, wings, body surfaces, feathers and cloacae were visually examined through a magnifying lens for ectoparasites. Haemoparasitic and coproscopic study along with postmortem examination of gastro-intestinal tract was conducted for endoparasites. Overall prevalence of ectoparasites was higher (66.1%) than endoparasites (33.7%). *Menopon gallinae* (Lice), *Columbicola columbae* (Lice), *Ascaridiagalli* (Round worms) and *Raillietina magninumida* (Flat worms) were noted as 11.2%, 54.9%, 11.2% and 22.5%, respectively. Discrimination in prevalence were found associated with sex of pigeon. Prevalence of ecto-parasite were more in male (86.6%) than female (51.1%) pigeons and endo-parasites was higher in females (36.5%) than male (29.9%) pigeons. The pigeons which were reared under-mix birds rearing type have been noticed to be have more kind of parasites as compare to those pigeons who were reared singly. In the light of these findings, it is recommended that pigeons should be reared without mixing them with other birds.

**Keywords:** Prevalence, Domestic breed, Pigeons, Pakistan
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**TITLE**

FAMACHA System As a Tool if Targeted Selective Treatment

*Haemonchus (H.) contortus* is the most devastating gastrointestinal nematode for small ruminants because of its economic significance. For control of *H. contortus*, traditional practices are adopted to deworm the entire flock according to the recommended schedule even when only a single animal shows the signs of haemonchosis. There are many reports of development of anthelmintic resistance in small ruminants due to excessive and un-necessary use of commercial anthelmintics. In the present scenario of anthelmintic resistance, there is need of targeted selective treatment (TST) to avoid anthelmintic resistance and to maintain the population in refugia. The classic example of TST is the FAMACHA system which is an eye color chart with five color categories and it is named after its originator Dr. Faffa Malan (FAffa MALan CHArt). This system is used in such a way that the color of the mucus membranes of lower eyelids of sheep is matched with the colors on FAMACHA card and thus it is given the score “1” which means healthy and “5” which indicates severely anaemic animal. In FAMACHA system, only those animals are treated which fall in the categories of “4” and “5” or in other words they are in danger of dying due to anaemia caused by *H. contortus*. The eye color chart has advantages that only 20-30% or less animals are treated, ultimately treatment cost is reduced and development of anthelmintic resistance is slowed down by keeping a portion of parasites in refugia.

**Keywords:** FAMACHA, Targeted selective treatment, *Haemonchus contortus*
Role of Wild Birds in Distribution of Toxoplasmosis

Toxoplasma (T.) gondii is intra-cellular apicomplexan protozoa with a cosmopolitan distribution. In Pakistan reported prevalence of toxoplasmosis is ranged from 11.33% to 29.45%. This plays an important role in reproductive disorders leading to abortion. Toxoplasma gondii affects warm blooded animals including humans and birds. Almost one third human population is infected by this. Among intermediate hosts of T. gondii avian birds are most important as they act as source of infection for feline which prey on these birds and shed the oocysts in the environment. Annually, about billions of birds are being preyed by cats. Ground feeding birds play role in the contamination of soil too. Toxoplasmosis is considered as source of mortality in some avian species. In humans, symptoms include: sever illness, psychiatric disorders, preeclampsia, abortion encephalitis, behavioral changes, neuropsychiatric disorders, brain abscesses, ocular problems, fetal abnormalities and death in immunocompromised hosts. Main source of human infection is ingestion of raw /undercooked meat, contaminated vegetables, fruits and water. Presence of T.gondii antibodies can be identified using Sabin-Feldman dye test (DT), enzyme linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), histological examination, isolation of parasite and modified agglutination test (MAT). Seroepidemiology of T.gondii will be a useful tool to evaluate environmental contamination with T.gondii and can be used to determine the risk of public health concern in association of environment and intermediate host. 

**Keywords:** Toxoplasma gondii, Wild birds, MAT, Seroepidemiology
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TITLE

Climate Change Impact on Parasitic Infections

Global warming had caused drastic changes in the environment from 1906-2005 by elevating the temperature up to 0.74°C, per year sea level rise up to 2mm, 7.4 % decrease in the ice extents of Arctic sea and diminishing of glaciers and snow covers. Now-a-days, changes in the climate are quicker as that happened in last 1000 years. According to the United Nations Intergovernmental Panel, global temperature will increase from 1.8°C -4.0°C and sea level will increase 18-59 mm in the coming 90 years. Droughts and floods will also compliment global warming. Climate change (CC) is among the main factors which affect the ecosystem. Climatological variation and ecological perturbation have been pervasive drivers of faunal assembly, structure and diversification for parasites and pathogens through often recurrent events of geographical and host colonization at all scales of Earth history. Host–parasite systems are ubiquitous. Understanding the factors that generate, maintain and constrain these associations has implications for broad ecological and environmental issues including the dynamics of EIDs, biological control, biological introductions and invasions, and biotic responses to climate change. The frequency distribution of vector-borne diseases is considered surging as a result of CC due to climate compatibility for vector growth, increase microbial growth and decreased resilience in host leading to spread of diseases. Factors favoring the animal disease burden include: virulency of pathogens, their routes of transmission and environmental compatibility. We can assess the risk potential of the disease by pathogen identification and can forecast and map the disease risk areas and for target specific implementation of control strategies. Disease modeling may assist in impact assessment, mitigation and adaptation of the climate change favored parasitic infections.
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TITLE

Serodiagnosis of anti-Toxoplasma gondii antibodies among Renal Failure patients Visiting Different Hospitals of Lahore

Toxoplasmosis caused by *Toxoplasma gondii* is an opportunistic infection. The infection is mostly asymptomatic in healthy individuals but in immunocompromised people, the parasite can become widely disseminated causing severe toxoplasmosis. Renal failure patients are at great risk for infecting with *Toxoplasma*. Present study was aimed to investigate anti-*Toxoplasma* IgG antibodies among renal failure patients undergoing hemodialysis from different hospitals of Lahore. A total of 80 renal failure patients undergoing hemodialysis and 40 healthy subjects were included for present study. Questionnaire survey was done to assess general health status and risk factors for *T. gondii* infection. Blood samples were taken from all study respondents and sera were separated for further analysis. The presence of serum IgG antibodies against *T. gondii* was determined by commercially available ELISA kit. Results showed that overall prevalence among study respondents was 43% with seroprevalence rate of 47% in case and 35% in control respondents. Age wise comparison was also done that showed respondents who belong to elder age group (60-75) were more seropositive (83%) for anti-*T. gondii* IgG antibodies as compared to younger study subjects. Gender wise comparison was also made and it was found that female respondents had more prevalence rate (67%) for *T. gondii* infection than male respondents (33%). Analysis of present data showed that seroprevalence rate was more in those respondents who were illiterate, had more kids, low socioeconomic status and poor hygienic conditions. It was found that renal failure has significant association (*P* < 0.05) with prevalence of *T. gondii* infection. More studies were recommended on susceptible groups for creating awareness among general population and for better public health.
There is considerable evidence that some anthelmintics, including ivermectin, upregulate the transcription levels of ABC transporters in nematodes; however, such an effect has not been studied for monepantel (MPL). This study investigated the effects of MPL on transcription of ABC transporters in two MPL-susceptible isolates of *Haemonchus contortus*. Larvae were exposed to MPL at two concentrations (2.5 and 250 µg/mL) for periods of 3, 6 and 24 h. Transcription levels of 16 ABC transporter genes were measured at the end of the incubation periods. The phenotypic consequences of MPL exposure were also measured. Multiple ABC transporter genes showed significantly higher transcription in both worm isolates following exposure to MPL at 250 µg/mL for 3, 6 or 24 h, particularly the P-glycoprotein genes pgp-11, pgp-12 and pgp-14. In contrast, there was only a single instance of low-level up-regulation following exposure to MPL at 2.5 µg/mL. Larvae exposed to MPL at 250 µg/mL showed an increased efflux of rhodamine-123 and a proportion of the larval population showed an ability to tolerate higher concentrations of ivermectin in migration assays. There was no increased tolerance to ivermectin following pre-exposure to MPL at 2.5 µg/mL. The increased transcription of multiple transporter genes, increased R-123 efflux, and subsequent ivermectin tolerance in a proportion of the larvae following exposure to MPL at 250 µg/mL, suggests a protective role of ABC transporters across different chemical entities, and a possible interaction of MPL with nematode ABC transporters.
**TITLE**

Distribution of Ixodid Tick Species and Associated Risk Factors in distinct Temporal Zones of Khyber Pakhtunkhwa Province, Pakistan

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Distribution of various Ixodid tick species and risk factors associated with tick infestation and burden levels were studied in bovine from three distinct temporal zones of Khyber Pakhtunkhwa (KPK) province of Pakistan. Twelve hundreds ticks were collected from four hundreds animals comprising of two hundred and fifty cattle and one hundred and fifty buffalos. Descriptive statistics with Pearson’s Chi-square test and regression model were applied to analyze the data. The results of study found *Rhepicephalus* the most prevalent genus followed by *Heamaphysalis*, *Hyalomma*, *Dermacentor* and *Amblyomma* with the prevalence of 78.51%, 10.33%, 10.08%, 0.66% and 0.41%, respectively. On species basis, *Rhepicephalus (Boophilus) annulatus*, *R. (Boophilus) microplus*, *Heamaphysalis aciculifer*, *R. appendiculatus*, and *R. decoloratus* were noted 41.6, 18.4, 9.8, 8.25, and 6.8% respectively, whereas least prevalence was noted as 0.41% in case of *A. pomposum* and *D. circumguttatus*; 0.25% was shown by *D. rhinocerinus*, *Heam. excavatum*, and *H. impeltatum*; 0.16% exhibited by *Heam. boqui* and *R. distinctus*; and 0.08% displayed by *Heam. parmata* and *H. egyptium*, *H. rufipes*, *R. longus* and *R. parvus*. Risk factor analysis namely housing type, tick control, age and sex of animal presented significant (P <0.05) association with tick infestation and burden while type of breed showed significant association with tick infestation but was non-significant with tick burden. Topography presented inverse behavior to that of breed with tick burden and tick infestation. On the other hand, geo-location was only factor exhibiting non-significant (P>0.05) association with both dependent variables. The study concluded that *Rhepicephalus* was the most prevalent Ixodid genus whereas, presumed risk factors were strongly associated with tick infestation and tick burden.
Cloning and Sequence Analysis of Highly Conserved *E. acervulina* Antigen

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Total RNA was extracted from sporulated oocysts of *E. acervulina* by the single step protocol. The RNA pellet was resuspended in diethylpyrocarbonate water and stored at -20°C until further use. The gene specific primers were designed based on cSZ-2 gene mRNA of *E. acervulina* of GenBank accession numbers Z26584 with addition of specific enzymes. The cDNA was synthesized and then, PCR was performed by reverse transcription (RT) reaction using primers according to corresponding NCBI mRNA sequence. The amplification product was recovered using “Takara Agarose gel DNA purification Kit version 2.0”, according to manufacturer's instructions and ligated to pMD-18T cloning vector. The resultant product was then, transformed into *E. coli* (DH5α) competent cells. One of the positive clones from Recombinant plasmids with inserts of the expected size identified with BamHI and EcoRI restriction enzyme digestion, followed by gel electrophoresis on 1% agarose gels. The complete *E. acervulina* cSZ-2 product were verified by sequencing, and confirmed to have 99% homology to the previously described *E. acervulina* cSZ-2 DNA fragment on NCBI. The sequence analysis confirmed that is highly potent and could be proved a highly immuno-dominant vaccine target against chicken coccidiosis.
The Methanolic extract (ME) and Chloroformic extract (CE) of *Coriandrum sativum* (C. sativum) were evaluated in comparison with ivermectin to cover the anthelmintic resistance in Salt Range sheep. A total of 60 sheep positive for nematodes were selected and randomly divided into 6 groups having 10 animals in each group. The animals of group A was kept Untreated control. Group B was given ivermectin at the dose rate of 0.2 mg/kg body weight subcutaneously. The animals of Group C and Group D were given the CE of *C. sativum* at the dose rate of 50 mg/kg body weight and 100 mg/kg body weight respectively. The Group E and Group F were given the ME of *C. sativum* at the dose rate of 50 mg/kg body weight and 100 mg/kg body weight respectively. Fecal eggs per gram (fecal EPG) tests were carried out on day 7, 14 and 28. The percent efficacy of ivermectin was 81.4%, 87.17% and 92.6% on day 7th, 14th and day 28th respectively. The percent efficacy shown by the CE at maximum dose (100 mg/kg body weight) was 10.7%, 17.72 and 24.41 on day 7th, 14th and 28th respectively. The percent efficacy of ME given to Group F was 44.76%, 56.27% and 60.69% on day 7th, 14th and day 28th respectively. From this study it was concluded that ME of *C. sativum* has maximum anthelmintic effect at the highest dose used in the study.

**Keywords:** Methanolic extract, Chloroformic Extract, *Coriandrum sativum*, anthelmintic resistance, salt range sheep
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Ticks and tick-borne diseases (TTBD) affect livestock production therefore hinder efforts to maximize the return on investment of inputs for animal agriculture, especially in production systems located in tropical and subtropical parts of the world. The concept of precision agriculture involves the use of exact management and control technologies to realize substantial savings in inputs by making use of newly available technologies such as global navigation satellite systems, geographic information systems, automated agricultural machinery, high-resolution image systems, sophisticated sensors, automatic control, and robotics. Under non-intensive farming, precision livestock production considers all agroecosystem components to increase efficiency and reduce environmental impacts. These efforts can be complicated when wildlife share the landscape with livestock and are alternate tick hosts or TBD reservoirs. A component of our research program involves the adaptation of precision livestock farming systems to address the problem with TTBD in cattle production. Drones and robotics have been tested for their ability to assess the risk for tick infestation across pastures. Remote sensing has been applied to understand the intricacies of the livestock-wildlife interface compromising TTBD control and eradication efforts. However, the benefits of integrating precision agriculture technologies to TTBD management remain to be fully realized.
Ticks and tick-borne diseases (TTBD) affect livestock production therefore hinder efforts to maximize the return on investment of inputs for animal agriculture, especially in production systems located in tropical and subtropical parts of the world. The concept of precision agriculture involves the use of exact management and control technologies to realize substantial savings in inputs by making use of newly available technologies such as global navigation satellite systems, geographic information systems, automated agricultural machinery, high-resolution image systems, sophisticated sensors, automatic control, and robotics. Under non-intensive farming, precision livestock production considers all agroecosystem components to increase efficiency and reduce environmental impacts. These efforts can be complicated when wildlife share the landscape with livestock and are alternate tick hosts or TBD reservoirs. A component of our research program involves the adaptation of precision livestock farming systems to address the problem with TTBD in cattle production. Drones and robotics have been tested for their ability to assess the risk for tick infestation across pastures. Remote sensing has been applied to understand the intricacies of the livestock-wildlife interface compromising TTBD control and eradication efforts. However, the benefits of integrating precision agriculture technologies to TTBD management remain to be fully realized.
Poster Presentations
Morphology and Characterization of Vector Babesia from Different Samples Collected from District Kasur

Bovine Babesiosis is a consistent threat to the livestock of Pakistan inflicting a considerable economic loss every year. The study of ticks serving as the vector of this disease is insufficient in this region. The current study had conducted to understand the abundance of major vector of bovine babesiosis in district Kasur, Punjab, Pakistan. A total of 100 cows and 100 buffalos were sampled and total of one hundred and twenty five ticks were collected from each group of animals. The collected ticks were morphologically identified and characterized through stereomicroscopy on the basis of their sex and species. In our study, *Boophilus microplus*, *Rhipicephalus e. evertsi* and *Haemaphysalis leachi* were identified as the major species of ticks responsible for transmitting babesia. Among sampled cows the percent relative abundance of *Boophilus microplus*, *Rhipicephalus e. evertsi* and *Haemaphysalis leachi* respectively. Likewise *Boophilus microplus* was also appeared with highest percent relative abundance in sampled buffalos. Moreover, the 53% of sampled cows and 66% of sampled buffalos were diagnosed to be diseased showing a significant relationship between disease and its transmitting agent. The results of this study shows that the *Boophilus microplus* as the major vector of Babesiosis in cattles.
This study was undertaken to elucidate the prevalence of ovine Babesiosis in Kashmir. A total of 691 sheep reared at different places of central Kashmir and presented to Veterinary Clinical Services Complex (VCSC), Faculty of Veterinary Sciences and Animal Husbandry, Shuhama (SKUAST Kashmir), Srinagar were screened from March 2016 to June 2017 for the prevalence of ovine babesiosis. A total of 185 cases of ovine babesiosis were diagnosed from 691 sheep screened. The prevalence rate was estimated 26.77%. The diagnosis was based on clinical manifestations, presence of ticks, demonstration of Babesia piroplasms on blood smear examination, and by PCR. The cases comprised of cross bred sheep (91.35%), Corridale (8.11%), South Down (0.54%) and Bakerwal (0.00%) breed. The disease was more predominant in females (78.37%) than males (21.62%). The seasonal prevalence of the disease was highest in summer (42.70%), followed by spring (38.91%), autumn (11.35%) and lowest in winter (7.03%). Adult sheep in the age group greater than 12 months (58.37%) were mostly infected with babesiosis compared to sheep of 0-3 months (24.32%), 3-6 months (8.65%) and 6-12 months (8.65%). Haemaphysalis ticks were identified as the vectors for the transmission of ovine babesiosis in the study area.
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TITLE
Seroprevalence of Toxoplasmosis in Female Breast Cancer Patients in Lahore

Toxoplasmosis is a zoonotic infection of humans and animals, caused by the opportunistic protozoan Toxoplasma gondii. Infection in pregnant women may lead to abortion, still birth or other serious consequences in new born. Infection in immunocompromised patients can be fatal if not treated. On average, one third of people are chronically infected worldwide. Cancer remains a leading cause of death, responsible for approximately 13% of global deaths. The prevalence of human infection with Toxoplasma gondii has been increasing day by day. Thus, the objective of present study was planned to determine the prevalence of anti-Toxoplasma gondii antibodies in breast cancer patients by examining the seropositivity and serointensity rate of Toxoplasma gondii. By using ELISA kit the seroprevalence of toxoplasmosis was analyzed. To find out the risk factors, all the information was collected with the help of questionnaire. 130 blood samples were collected from Sir Ganga Ram hospital Lahore out of which 90 samples were case and 40 samples were control. In a 90 samples of case 42 were seropositive and 48 were seronegative. In a control group out of 40 samples of case 14 were seropositive and 26 were seronegative. Among breast cancer and non breast cancer female’s prevalence rate was 46.6% and 35% respectively Overall prevalence in breast cancer female population in Lahore was found 46%. To reduce the infection rate in a local population of Lahore, health education and public awareness is needed.
Abstract: This research work was designed to study the prevalence of anaplasmosis among cows and buffaloes in three Tehsils (Mardan, Lundkhwar and Katlang) of district Mardan, Khyber Pakhtunkhwa province, Pakistan. A total of 600 blood samples were collected and examined under Microscopic using Giemsa stained blood smears. Obtained results revealed an overall prevalence of parasites as 7.40% and 5.44% in cows and buffaloes, respectively. The overall seroprevalence of anaplasmosis in cows using cELISA was 32.66%. Females were found to be more susceptible 70/200 (35.00%) as compared to males 28/100 (28.00%). The prevalence was significantly higher 80/180 (44.44%) in cows than calves 18/120 (15.0%) ($P<0.05$). The seroprevalence in cows was significantly ($P<0.01$) higher in summer season in the district. The overall seroprevalence of anaplasmosis in buffaloes using cELISA was 23.66% (71/300). Females were found more infected (54/220, 24.54%) as compared to males 17/80 (21.25%). The prevalence was higher 50/190 (26.31%) in above two year buffaloes than the younger ones 21/110 (19.09%). The seroprevalence in buffaloes was significantly ($P<0.001$, $P<0.01$ and $P<0.05$) higher in summer, spring and autumn seasons in the district, respectively. It was concluded that anaplasmosis is widely distributed in district Mardan, Khyber Pakhtunkhwa, Pakistan. Further research should be undertaken for anaplasmosis control.
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<td>First record of occurrence of <em>Oestrus ovis</em> infestation in lamb from Poonch District of Azad Kashmir, Pakistan</td>
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Myiasis is the infestation of organ and tissues of human, wild, domestic and farm animals with maggots of myiasis causing flies. *Oestrus ovis* is very common among myiasis causing flies in sheep reported from several parts of the world. A lamb after sudden death was presented for post-mortem and expert opinion at livestock experimental station of Faculty of Veterinary and Animal Sciences, University of Poonch, Rawalakot, Azad Kashmir to know the cause of sudden death. Before performing post-mortem, detail history was taken from the shepherd. Lamb was keenly observed for external and internal abnormalities. The finding of post-mortem includes: mild ascites, congested spleen, and petechial haemorrhages on lungs, ocular and nasal discharge. All other examined organs were found normal. However, when skull was opened for brain examination, four larvae/maggots were found in sinuses of brain. Larvae/maggots were identified macroscopically on the basis of their stigmal plates shape and were identified as L4 stage of *oestrus ovis*. On the basis of these findings, it is concluded that death of lamb might be due presence of these maggots in sinuses. This is first reported evidence based case that showed the occurrence of *oestrus ovis* fly in geographic setting of district Poonch. Therefore, it is recommended to conduct wide-range surveillance throughout the Azad Kashmir to determine the frequency distribution, seasonal pattern of myiasis causing flies in lamb/sheep and other livestock species for devising control strategies.

**Keywords:** Lamb, Death, Brain, *Oestrus ovis*, Larvae, Poonch, Azad Kashmir.

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Caprines reared at Mountain Research Center for Sheep and Goat (MRCSG), Srinagar, Kashmir exhibited reduced efficacy to closantel and ivermectin (IVM) in Faecal Egg Count Reduction Test (FECRT). The results suggested that the overall efficacy for IVM was 83.54% and 90.00% on 7th and 14th day post-treatment, respectively and for closantel it was 68.35% and 86.25% on 7th and 14th day post-treatment, respectively. The pre-treatment faecal culture revealed *Haemonchus contortus*, *Ostertagia circumcincta* and *Trichostrongylus colubriformis* as predominant strongyles, however, in post-treatment samples, *H. contortus* was only observed. The survey indicated that the Gastro-intestinal nematodes (GINs) especially *H. contortus* of goats on MRCSG have developed multiple anthelmintic resistance to closental and IVM and the condition is alarming on the farm.

Keywords: Closental. FECRT. GI Nematodes. Goats. IVM.
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**TITLE**

*In vitro*, evaluation of Azadirachta indica and Melia azedarach leaves against *Rhipicephalus (Boophilus) microplus*

Increasing incidence of ticks and tick borne diseases let to execution of this study. The crude methanolic extract of *Azadirachta (A.) indica* and *Melia (M.) azedarach* leaves were evaluated for their efficacy against *Rhipicephalus (Boophilus) microplus* through adult immersion test and larval packet test. The adult mortality percentage for reproductive index, percentage inhibition of ovi-position, hatching and larval motility were studied at concentration of the extracts i.e. 15, 30, 60, 120 mg/ml. At the highest concentration (120mg/ml) 75.03 and 85.05 % tick mortality was noted for *A. indica* and *M. azedarach*, respectively. 53.5 and 62.5 % inhibition of ovi-position was recorded at the highest concentration (120mg/ml). Larvae treated with all the tested concentration of *A. indica* and *M. azedarach* showed significant mortality (p<0.001) than that of control. Thus, study showed that *A. indica* and *M. azedarach* has a promising effect as a acaricidal and may be recommended for prevention and control of ticks’ population.

**Keyword:** Anti-tick activity, *Azadirachta indica*, *Melia azedarach*, methanolic extract, in-vitro trials
The present study was aimed to determine the prevalence and associated risk factors of coccidiosis in sheep and goats in Dera Ghazi Khan. A total of 752 fecal samples (n=376 sheep; n=376 goats) were collected, and examined coprologically. The prevalence of coccidiosis in sheep (52.92%) was significantly higher ($P<0.05$) compared to goats (44.41%). Eimeria infection was significantly higher ($P<0.05$) in female animals compared to males. The prevalence of coccidiosis was significantly higher ($P<0.05$) in ≤6 month age group sheep and goats followed by >6 months but <1 year, and above 1 year age groups. A significantly higher ($P<0.05$) prevalence was found in stall feeding and confined animals compared to grazing and animal having outdoor access. Prevalence of coccidiosis was significantly higher ($P<0.05$) in animal with poor and weak body condition compared to healthy animals. A strong association ($P<0.05$) was observed between fecal score and prevalence of *Eimeria* infection. Peak prevalence was observed in August while the lowest in October. The most abundant Eimeria spp. in sheep was *E. ovinoidalis* (56.78%) followed in order by *E. ahsata* (47.23%), *E. parva* (35.67%), *E. intricata* (30.15%), *E. faurei* (26.63%) and *E. pallid* (19.09%). In goats, the commonest Eimeria spp. was *E. ninakohlyakimovae* (68.86%), following by *E. aliievi* (59.88%), *E. arloingi* (53.29%), *E. caprina* (46.70%) and *E. birei* (22.15%). It was concluded that various Eimeria spp. were quite prevalent and several risk factor affect the occurrence of coccidiosis in study area.
Tick control is practiced in a variety of ways in different livestock systems. Treatment of livestock with acaricides to kill attached ticks is the most widely used control method. But unfortunately, excessive and unnecessary use of acaricides has led to the development of acaricide resistance along with many side effects. There are other common tick control strategies in practice like grooming, pasture management, biological control, genetic manipulation, vaccination and herbal approach. Grooming is the manual removal of ticks, which is widely used in the developing world, although it is rare in extensive systems. Pasture spelling (depopulating pastures while free-living ticks die because of a shortage of available hosts) is well known as a means to control ticks. In biological control of ticks, candidate methods include ants, predatory mites, chickens, parasitoid wasps, Bacillus thuringiensis and entomopathogenic nematodes. Development of ‘cattle lines’ or a breed with enhanced, genetically based disease resistance is an especially attractive prospect. Good examples of exploiting genetic resistance to livestock diseases in general and parasites in particular, have been described for resistance of Bos indicus to cattle ticks. Vaccines so far commercially available were developed to be effective against Boophilus microplus and are based on the tick midgut protein Bm86. A commercial product based on the Bm86 antigen is TickGARDPLUS. Exploration of the possibilities to use botanical acaricides for the control of ticks has been identified as one of the future options. The use of herbal preparations among the rural folks is gaining importance because of their therapeutic value, their local availability and cost effectiveness.

**Keywords:** Ticks, control strategies, livestock
Ticks and tick-borne diseases (TTBDs) are considered to be of most significance due to their economic importance for livestock enterprises particularly in resource poor communities. They affect 80% of the world cattle population with high prevalence and cause huge production losses particularly in tropical and subtropical regions of the world. TTBDs are ranked number fourth in infectious livestock diseases while tick-borne diseases (TBDs) are considered to be the most significant vector borne diseases. East Cost Fever (ECF) and anaplasmosis are important TBDs of livestock. ECF is endemic in many countries of Southern, Central and Eastern Africa which is caused by a protozoan parasite *Theileria parva* and transmitted by the tick *Rhipicephalus appendiculatus*. The calves are severely affected by this diseases resulting in 40-80% annual mortality. On the other hand, *Anaplasma marginale* causes bovine anaplasmosis which is so important economically that 100 million US$ were spent annually along with the death of 50,000-100,000 cattle heads in USA alone. Many losses are directly caused by ticks in the form of ‘tick worry’ during their attachment to the animal skin, blood loss, irritation and hide damage, general stress, injection of toxins and production losses in terms of reduction in milk and meat, etc. Indirect losses include the depression of immune system and ultimately transmission of disease causing pathogens. Many factors contribute to the spread of TBDs including inadequate resources, veterinary infrastructure, management practices and production systems. These factors invite the researchers to find some alternate ways to control ticks, particularly for livestock dependent systems of developing and underdeveloped countries.

**Keywords:** Ticks and Tick-Borne Diseases, *Rhipicephalus*, Anaplasmosis
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The purpose of this study was to evaluate the efficacy of ivermectin and fipronil local application against frequently received clinical cases of scaly face mite i.e. Knemidocoptes pilae in budgerigar (Melopsittacus undulates). Evaluation was performed on two equal size groups of budgerigars infected with K. pilae. Fipronil and ivermectin spot on were administered on neck region in respective group on days 0, 7, 14. It was observed that ivermectin resulted in rapid healing by day 7 while fipronil initiated healing by day 14. Both ivermectin and fipronil led complete recovery in all infected birds till day 21.
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Parasites as Biological Tags for Monitoring the Health of Water Bodies

Freshwater ecosystems are under increasing global pressure (Verhoeven et al., 2006) and freshwater organisms are generally much more endangered than terrestrial organisms (Dudgeon et al., 2005). Among those freshwater organisms many form our day to day food like that of fish. Besides containing protein and other nutrients such as vitamin D, fish contain a specific type of fat, omega-3 fatty acids, that may reduce the risk of developing heart disease and other medical problems. In Kashmir the number of native fish *Schizothorax* spp. Is decreasing and in order to understand the factors responsible for deteriorating the quality and quantity of fish present investigation was carried out on *Schizothorax esosinus*. A strong positive correlation was observed (Pearson’s correlation, r=0.9; P=0.038;) between total length of *Schizothorax* and degree of parasitism. Prevalence and mean intensity were positively and significantly correlated with different seasons (r=0.7, P<0.01 and r=0.9, P<0.01, respectively). The above findings will be useful in devising the appropriate control strategies for the Helminth in wild fish in Kashmir valley as well as in similar climatic zones of other parts of the world.

**Keywords:** - River Sindh; *Pomphorhynchus kashmirensis*; *Schizothorax*, Kashmir
Leishmaniasis is a parasitic zoonotic disease caused by obligate intracellular protozoan parasites of genus *Leishmania* and belongs to family *trypanosomatidae*. These parasites develop different forms of the disease including cutaneous, mucosal and visceral leishmaniasis. The range of manifestation depends on immune level of the host, type of parasitic species involved and inflammatory reactions. Leishmaniasis causes high morbidity and mortality in underdeveloped and developed countries. In the current era, research has been focused on advanced techniques for rapid and effective diagnosis of these parasitic infestations. Effective laboratory techniques are mandatory for exact diagnosis of the disease particularly in the patients those are immuno-compromised due to HIV. The current review focus on the present state of diagnostic tools based on microscopic observations, detection of antigens, serological analysis including indirect fluorescent antibody test, ELISA, immuno blotting test, direct and latex agglutinations, immunochromatic test, polymerase chain reaction, loop mediated isothermal amplification test, intradermal leishmania skin test and nucleic acid sequence-based amplification test.
Identification and Characterization of *Ancylostoma caninum* Antigens for Their Use in Immunodiagnosis

*Ancylostoma caninum* is a common canine hookworm that routinely infects gastro-intestinal tract of dogs causing gastric disturbances. It has high zoonotic implication causing cutaneous larva migrans (CLM) and eosinophilic enteritis (EE). Prevalence study of gastrointestinal nematodes in dogs was carried out at Referral Veterinary Polyclinic, Indian Veterinary Research Institute, from September 2011 to March 2012. Out of 434 dogs screened during study, 90 (20.74%) dogs were positive for *A. caninum*, 64 (14.74 %) dogs were positive for *T. canis* and 36 (8.29%) were found to harbor mixed infection of *A. caninum* and *T. canis* with overall prevalence of GI helminthes in dogs as 43.77%. Pups showed high prevalence of parasitic infection than adults (69.36% vs. 37.28%). The highest and lowest prevalence of *A. caninum* was observed in the months of December (23.07%) and September (16.90%), respectively. Characterization of adult somatic, adult excretory/secretary and larval somatic antigen of *A. caninum* was also carried out in this study. SDS-PAGE analysis of adult somatic antigens showed 13 visible bands ranging from 235.5 to 11.2 kDa with 6 prominent bands, adult ES antigens showed 8 visible bands ranging from 102.0 to 12.0 kDa with 5 prominent bands and larva somatic antigens showed approximately 14 bands ranging between 6.0 and 195.3 kDa with 8 prominent bands. Western blotting of adult *A. caninum* somatic, adult *A. caninum* excretory/secretory and *A. caninum* larval antigens showed 5, 4 and 5 immuno-reactive bands of which 2, 3 and 3 were cross reacting with *T. canis* hyperimmune sera (HIS), respectively. Molecular weights of proteins specific to *A. caninum* in adult somatic antigens were 104.4, 70.0 and 29.5 kDa, while bands of 48.0 and 11.2 kDa were cross reactive with *T. canis* HIS. Similarly, in adult-ES antigens the molecular weights of specific protein was 16.4 kDa, while bands 102.0, 53.7 and 33.0kDa were cross-reactive with *T. canis* HIS. Western blotting of larval somatic antigens depicted 5 bands with molecular weights of 195.3, 55.03, 42.06, 37.06 and 11.7 kDa as immune-reactive, out of which 2 bands with molecular weights of 55.03 and 42.06kDa were *A. caninum* specific.
A study was undertaken to assess the effect of supplementation of exogenous fibrolytic enzymes (EFE) cocktail and *Artemisia absinthium* L. herb alone and in combination as feed additives on *in vivo* anthelmintic activity in lambs. Twenty crossbred lambs randomly divided into four groups were fed for a period of 90 days on oats straw based complete feeds supplemented either with EFE cocktail (T₁) @ 6 g/kg DM or wormwood herb (T₂) @ 4.5% of DM or in combination (T₃), whereas the complete feed without any supplementation served as control (T₀). Faecal eggs per gram (EPG) count and haematological and biochemical parameters of animals in all the groups at start and subsequently at monthly intervals were investigated. Faecal egg count reduction (FECR) assay was obtained by calculations. The feed additives when supplemented in combination (T₃) had significant (*P*<0.01) effect for %DCP and DE. Feed additives supplementation had non-significant effect, but there were significant (*P*<0.01) effect of the period in wormwood herb supplemented groups on overall mean EPG counts. Wormwood herb supplementation (T₂ and T₃) had significant effect on overall means (*P*<0.05) as well as at each feeding period (*P*<0.01) of %FECR. The haematological and biochemical profiles of feed additives supplemented lambs revealed better physiological health status compared to un-supplemented animals. It is concluded that inclusion of wormwood herb as feed additive in complete diet has better anthelmintic activity compared to EFE cocktail supplemented alone in sheep.

**Key words:** EPG, FECR%, Exogenous enzymes, Haemato-biochemistry, Sheep, Wormwood
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Theleriosis or East Coast Fever is a vector borne disease caused by ticks of Genus Hyalomma and Rhipicephalus. Most common blood protozoan identified in Pakistan are viz; Thelaira annulata and Thelaira parva but now a days a third form of disease has been recorded with sudden onset of disease including lymphadenopathy, high rise of fever and proptosis leading to fatal result of death. The infected animals at local farm were surveyed with parasitological techniques including light microscopy which proved to be quite helpful for the diagnosis and presence of theleria in RBcs of infected animals. Ten animals were declared infected on the basis of history and microscopy. Initially the treatment was started with Imidocarb (3 mg / kg body weight) and buparvaquone (2.5 mg/kg body weight) as and when diagnosed and later oxytetracycline (20 mg/kg body weight) was added in regime. Nine animals were cured but one animal showed a poor response to the standard recommended therapy and it died after 24 hours of exhibition of disease signs despite of initiation of treatment. The death of exotic cow was caused by Acute Theleriosis, which is new to be documented and further molecular techniques are required for its quick, timely and accurate diagnosis and effective therapy.
Molecular Epidemiology of Small Ruminant Theileriosis in Rahim Yar Khan district, Punjab, Pakistan

Piroplasmosis, caused by tick borne haemoproteozoa viz; Theileria and Babesia (Apicomplexa: Piroplasmida) is considered as a summer nightmare of small ruminants in Pakistan. It has been reported to cause annual economic losses of several billion US dollars in terms of cost of production loss and control measures worldwide. The present study purposed to reveal the molecular epidemiology of theileriosis (*T. ovis*) in the sheep and goat populations in Rahim Yar Khan district, Punjab, Pakistan. To this end, 384 blood samples were collected from sheep and goats through purposive selection method (severely infested with >20 ticks) from May-August. Relevant information from the screened animals was recorded on a predesigned questionnaire using participatory epidemiological techniques. Microscopic examination of the Giemsa-stained blood films revealed overall 0.25% (1/386) prevalence of *T. ovis*, whereas PCR setup was performed by using species specific primers. PCR results indicated 2.59% (10/386) samples positive for *T. ovis*. Although statistically insignificant, the prevalence of *T. ovis* was higher in sheep (3.19%), females (1.81%) and young-stock (1.55%) than goats (2.02%), males (0.772%) and adults (1.03%), respectively. Although smaller fraction of small ruminant population was found positive in the present study; however detailed vector screening studies for pathogens can be done on molecular grounds in the study district.
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<td>Echinococcosis: An Important Zoonotic Disease Transmitted by Tapeworm</td>
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Meat is an important component of our food chain. Despite of providing various vital nutrients it can be a source of several diseases. *Echinococcus granulosus* is an important and one of the smallest tapeworms of dog whose larval stage cause a lethal zoonotic disease of public health importance called as hydatidosis. Intermediate host of the tape worm include cattle, buffalo, sheep, goat and humans as well. The disease is characterized by formation of cysts of various sizes ranging from pea size to the size of a tennis ball. These cysts can be formed on any internal organ of the body but mostly it affects liver, spleen, heart, brain and lungs. *E.granulosus* is a small tapeworm of 3-6 mm size and have 3 segments. The genus *Echinococcus* has 6 species but the most pathogenic one is *E. granulosus*. The disease is cosmopolitan in distribution and prevalent in America, Australia and Middle East but it mostly abundant in developing countries like Iraq, Lebanon, Syria and Pakistan. The prevalence of disease in domestic animals in Pakistan is 6.67%. Despite of causing disease in humans it also causes a huge economic loss to the meat industry as well by condemnation of meat. Disease don’t show very pronounced signs and symptoms so it is very difficult to diagnose. It can only be confirmed by observing cysts after necropsy. The disease occurs in humans when they eat raw or undercooked meat containing the larvae of the worm. In Pakistan, the cases of human echinococcosis are also reported so it is the need of the hour to control the disease and to devise a strong strategy to avoid the occurrence of this zoonotic disease in humans.

**Keywords:** Echinococcosis, Hydatid cyst, Cystic echinococcosis, zoonosis.
Vector borne diseases are main threat to the world as they spread quickly and cause severe illness. They are known as major cause of morbidity and mortality in animals and humans. Arthropods including mosquitoes, ticks, mites, fleas, flies act as vector for transmission of diseases. Some vector borne diseases require amplifying host or reservoirs for completion of their transmission cycle. So, Dogs and Cats act as reservoirs in many vectors borne diseases including leishmaniasis, anaplasmosis, borreliosis, bartonellosis, Q- fever, Bartonella species. Feline vector borne diseases (FVBDs) have emerged in recent years and are gaining importance as diseases of public health concern under one health approach. During recent years FVBDs are widely distributed and their prevalence is increasing day by day due to changing climate. Environmental factors and human association with their pets along with climate change effect on vector abundance are the basic reasons of increase in feline vector borne diseases. Work on FVBDs is less reported so little data is available regarding this. Cats as compared to dogs are less exposed to arthropods. Their habitat and behavior minimize the arthropod infection risk. Detection of FVBDs agents can be challenging due to presence of unspecific clinical signs in healthy cats. Prevalence of feline vector borne pathogens in cats can be diagnosed by using conventional methods including conventional staining, serological tests such as immuno fluorescent antibody technique (IFAT), indirect immunofluorescent assay (IFA) and by using molecular technique like Polymerase chain reaction (PCR). PCR is a very effective technique for detection of organisms, to assess the role of subclinical infected cats and dogs in transmission of zoonotic diseases.

Keywords: Feline vector borne diseases, Ticks, Reservoir host, Climate, PCR
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**About 80%** diseases are zoonotic in nature. Too much diseases belonging to parasitic origin like leishmaniasis toxoplasmosis, cryptosporidiosis, and giardiasis. Leishmania is a protozoal disease caused by vector sand fly. 500 plus species of sand fly in the world but only 30 phlebotomine species are transmit the disease. Serious zoonotic threat for the human being after malaria. Required temperature for their survival and cause infection at 15-38°C and 70% relative humidity. In recent time about 88 countries are prevalent with Leishmania. About 1.5-2 million new cases are record per year. Over 1 billion people living in endemic areas at high risk of infection. About 500000 new cases of visceral Leishmania are recorded. About 22000 people were affected annually in northwest region of Pakistan. Cutaneous and mucocutaneous cases are high due to animal and human closeness to each other and occur in rural areas of Pakistan. In rural areas of Pakistan no safety measures should be adopted by farmers and population. Animal and people are close to each other due to that reason the sand fly population increases on the dunk of the animal and favorable for the life cycle of the Leishmania because host are easily approachable. This infection transmits to the animal and human and animal to human through wound of the Leishmania occur on the skin. Reduce the population of the vectors by applying the insecticides. Affected person do not let down the milk of the animals and affected animal separate from the herd. Sleep outside the door to wear full sleeves shirt and pant. Early diagnosis and early treatment better for the health. To educate the people and run extension programs. Different molecular methods successfully evaluated for Leishmaniasis like PCR-based assays the main molecular diagnostic tool for researchers and health professionals.

**Keywords:** zoonotic, leishmaniasis, PCR-based assays.

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**Leishmaniasis Serious Zoonotic Threat for the Rural Population of Pakistan**
Main pillar of Parasitological research is proper identification and classification of species using reliable morphological keys. However, due to some limitations in conventional taxonomical method there is need of some new and simple method for identification. Mosquito act as vector for many diseases such as malaria, arboviruses, filariasis to humans and animals. Based on morphological features mosquitoes are divides into different species. In class Insecta 150 species are public health important and act as vector. Among them genera *Anopheles*, *Aedes* and *Culex* are involved in transmission of diseases which cause morbidity and mortality in humans. Proper identification of mosquitoes is necessary for control of vectors which has a direct effect on medical implications. In the past isoenzyme markers, cyto-genetics and morphological keys are used for identification. Nowadays molecular methods are used for identification with great accuracy. DNA barcoding is a technique which use the specific genetic sequences used as genetic marker for identification of specific characteristics based on that mosquitoes are divided into different species. Aim of this study will be use of mitochondrial gene COI during DNA barcoding for identification of mosquitoes so that a molecular phylogeny will be established and to check genetic divergence of public health importance.

**Keywords:** DNA barcoding, Mosquitoes, COI
**Non Chemical Control of Helminths in Ruminants: Alternate Solution for Alternate Worms**

Gastro-intestinal helminthes infection in animals causes a substantial loss in terms of productivity and constitutes serious economic losses in the world. Diversified approaches are being utilized for the effective control of parasitic infection in animals, particularly anthelmintics. Lavish and inappropriate use of anthelmintics has developed resistance against parasitic infections. In addition, elevated cost of anthelmintics therapy reflected the attention of scientists to find relatively cheaper and environment-friendly methods. In parasitized animals, reduction in immunity to fight against endo-parasites occurs due to trace element deficiencies. It is well known fact that animals possessing optimum levels of trace elements are likely less prone to parasitic infections. Apart from the managed forages, the existing agro-ecological zones of Punjab have different types of medicinal botanics naturally present in rangelands and are utilized as fodder by small ruminants. These medicinal botanics are rich in trace element contents and may act as a natural resource to improve the trace element deficiencies leading to immunity boost-up in general and against gastrointestinal parasitic infections in particular. The use of mineral rich plants will be a cheaper and sustainable (there is no chance to development of resistance) source of preventive therapy against parasites, bacteria and malnutrition for the economic poor community of Pakistan.

**Keywords:** Gastro-intestinal parasites, Trace elements, Forages, Phytotherapy
Coccidiosis is an important protozoan disease of poultry having great economic importance. There are huge economic losses in poultry sector due to this disease. In general, this is controlled by using the anticoccidial drugs in feed or drinking water, but this practice having constraints of emergence of drug resistance in *Eimeria* species against available anticoccidial drugs and their residues in poultry products. Vaccination has also been showing good results in controlling this pathogen but this practice may result in onset of outbreaks in case of poor management practices, particularly, in case of live vaccines. The use of plants and plants derived products such as essential oils has also been an appealing approach in the recent years but essential oils may have cytotoxic effect and thus results in damaging cell wall and cell membrane of host cells. Some essential oils also show anti nutritional characteristic with low growth rate and weakness in birds. Probiotics may be the good hope in the face of above discussed challenges in controlling coccidiosis because of having properties such as immune stimulator, action as antioxidant and important role in maintain the intestinal health. The aim of this review article is to provide a comprehensive account of the use of probiotics in poultry industry for controlling coccidiosis alone or in combination with the anticoccidials, vaccines and plant extracts.
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**TITLE**

Acaricidal Activity of *Nicotiana tobacum* Extracts in Cattle and Associated Risk Factors

*Nicotiana Tobacum* has been evaluated for its antiparasitic activity in different animals however, its acaricidal activity against ticks have not been evaluated yet. The present study was designed to determine factors affecting the prevalence of ticks in cattle in district Loralai of Balochistan and to evaluate the acaricidal activity of chloroform and methanol extracts of tobacco (*Nicotiana tobacum*). A total of 670 cattle of different breeds, age and gender were examined for tick infestation with overall prevalence of 21.49% in Loralai. Friesian was more infected (26.15%) as compare to non-descriptive (22%) and Sahiwal (12.80%) breeds. Similarly, cattle less than one year old were most infected (27.90%) followed by those between 1-2 year (26.88%); the least prevalence was in cattle more than 2 years of age (19.34%). Higher prevalence was noticed in female cattle (21.98%) as compare to male cattle (16.92%). Three concentrations of *Nicotiana tobacum* (12.5mg/mL, 25mg/mL and 50mg/mL) were prepared in chloroform and methanol. The acaricidal activity of these extracts was determined by egg laying index and percentage inhibition of egg laying. The decline in egg laying index was significantly more by chloroform extract (10.048%, 17.378% and 25.143%) as compare to methanol extract (6.367%, 13.152% and 20.827%). Hatchability of eggs in chloroform extract was less than that in methanol extract (67.5%, 43.5% 17% and 77.5%, 47.5% and 23%) respectively. We concluded that the prevalence of ticks in cattle is affected by their age, breed and gender and that chloroform extract of *Nicotiana tobacum* is more acricidal as compared to the methanol extract.
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The present study was designed to investigate the effect of *Embilica officinalis* tannins on cell mediated immune response in chickens especially and protective efficacy against avian coccidiosis. Cell mediated immune response was detected by classic toe-web assay by injecting T-cell mitogen, PHA-P (100µg/100µL/ chicken; intra-dermally) and inflammatory response was measured at 24 to 72 hours post PHAP injection by subtracting the pre-injection measurement from the post-injection measurement of toe web thickness. The highest response was seen at 24 hours post PHA-P injection both in the experimental and control groups followed by 48 and 72 hours; although the results were statistically non-significant. Chickens administered with *E. officinalis* derived tannins showed comparable lymphoproliferative response rather higher (@ 0.75gm/kg b. wt) in terms of swelling in comparison with the chickens administered commercial tannins. Graded dose dependent lymphoproliferative response was not observed neither in the commercial nor in *E. officinalis* derived tannins. Mean oocysts per gram of droppings (OPG) was significantly higher (P<0.05) in negative control group as compared to the birds of other experimental group as well as positive control group treated with commercial tannins @ of 1mg/kg of body weight. Lesion scoring (scale 0 to 4) of survived and dead chickens administered with *E. officinalis* derived tannins @ of 0.75mg/kg of b. wt. developed mild, moderate and sever lesion score up to 40%, 30% & 30% respectively while lowest caecal lesion score of the mild to moderate intensity as compare to the other groups. Chickens administered with *E. officinalis* derived tannins @ of 0.75mg/kg of b. wt also showed the significantly higher (P<0.05) weekly gain as compare to the negative control group but it was no significant to other groups. The difference in the organ body weight ratio of the lymphoid organ except thymus and bursa was statistically non-significant.

**Keywords:** Tannins, cell mediated immune, Coccidiosis, Chickens
Overall, seasonal and monthly prevalence of helminth parasites in cattle and buffalo in Kashmir valley, India

During the present study for two years on the epidemiology of gastro-intestinal helminths in cattle and buffalo of Kashmir Valley, a total of 18 helminths species were recorded. Nematode and trematode species were dominant helminths followed by cestode species. The overall prevalence of trematodes was found 53.96% & 49.64% in cattle & buffalo. Data on monthly incidence of helminth infection showed that higher infection of helminths occurred in the month of June, July and August. Regarding the seasonal fluctuation of helminths, trematode infection was highest (63.26%) in autumn and summer (67.80%) and lowest in winter (47.51% & 26.92%) in cattle. While as in buffaloes, the highest infection was reported in summer (44.68% & 68.51%) and lowest in winter (21.73% & 52.94%). In case of cattle the cestode infection was maximum in summer (9.58%) & winter (13.04%). While as in case of buffaloes, the highest infection was found in summer (9.25%) and in autumn (9.52%). In both hosts, the lowest prevalence was found during winter (3.54% & 5.12%); autumn (4.16%) and in spring (8.00%) respectively. The highest gastrointestinal nematode infection in cattle was reported in summer (53.84%) of first year and again in summer (70.54%) of next year. The lowest infection in both the years in case of cattle was reported in winter (39.74% & 32.62%). In buffaloes, the highest nematode infection as per the gut examination was found in spring in both the years (60.00% & 54.05%) and the lowest infection was reported in winter (34.78% & 25.49%) respectively.

Keywords: Cattle; Buffalo; Helminth, Prevalence; Month; Season.
Nematodes infestation is one of the major problems of animals in Pakistan, causing huge economic losses in bovines. These are one of the major threats to livestock productivity. The anthelmintic effect of dried powder of ginger was evaluated against the gastrointestinal nematodes of cattle in District Rajanpur. A total 150 cattle positive for GIT nematodes were included in this study. These cattle randomly divided into 5 groups of 30 viz A, B, C, D and E, treated with Ginger @ 3 g/kg, 3.5 g/kg, 4 g/kg and Gurrh (placebo) @ 1 kg by per oral route, respectively. Fecal sample from each of cattle in all groups were collected directly from rectum at day 0 (pre-treatment) and then at day 14 (post-treatment) for EPG calculation by McMaster technique. The efficacy of ginger was calculated by using The Fecal Egg Count Reduction Test (FECRT). Anthelmintic efficacy of Group B, Group C, Group D and Group E were 86.20%, 91.39%, 89.65%, 87.05%. Ginger at dose rate of 3.5 g/kg, showed maximum EPG reduction. The current study highlights the effect of ginger against worms of cattle, and hence is a potential alternate for different allopathic medicines against which resistance has developed or is developing.
The objective of this study was to investigate, through cross-sectional survey, the distribution, types and prevalence of gastro-intestinal parasites affecting stray dogs in Assam India. Freshly voided fecal samples of 216 identified, stray, non-descript dogs of either sex and different age groups were collected and examined for coprological examination by direct faecal smear method; simple flotation and sedimentation techniques to detect parasitic oocysts and/or eggs. A total of 116 samples were found positive for helminthic eggs (nematodes and cestodes) while 100 (14.2%) were found to be positive for protozoan infections. Single parasite infections were more common than two or more infections. The data on the distribution of the various worm species in the positive dogs indicate that *Ancylostoma caninum* eggs were by far the most common. The other detected worm egg species and their respective frequencies were: *Dypilidium caninum*, *Spirocerca lupi* Toxocara canis, *Taenids*, *Toxocara leonina*, *Diphylobothrium latum*, and *Physaloptera canis*. The prevalence of helminth eggs was higher in puppies compared to adults. The prevalence of different species of helminths also varied in different age groups. The high prevalence of gastro-intestinal helminth parasites of zoonotic potential registered in the dogs indicates a potential risk to human health. The public health significance of the encountered parasitic infections is described and the prevalence is compared with the surrounding areas.

**Keywords:** Canines, Endoparasitic infections, Mathura, Prevalence, Public health significance, Zoonoses
A cross sectional study was conducted in domestic cross breeds lactating dairy cows. Mastitis was detected using the California Mastitis Test (CMT) and clinical inspection of the udder. For retrospective studies of mastitis in valley, the records from the past 10 years were studied at veterinary clinics, SKUAST-K of district Ganderbal and its adjacent areas. Data such as abnormal changes in the milk, mammary gland and CMT score were collected during animal examination. Depending on this clinical inspection and CMT results, cases were categorized as either positive or negative. The overall prevalence of mastitis for last ten years was calculated. The animals affected with mastitis were 140 out of 1001 in 2002-2003, 174 out of 1110 in 2003-2004, 158 out of 950 in 2004-2005, 114 out of 885 in 2005-2006, 181 out of 1210 in 2006-2007, 154 out of 1001 in 2007-2008, 122 out of 809 in 2008-2009, 118 out of 699 in 2009-2010, 199 out of 1101 in 2010-2011 and 477 out of 2998 in 2011-2012. The prevalence of mastitis in the present cross sectional study from 2012-2013 was found to be 16.89 per cent. Prevalence of bovine mastitis over the last 10 years was observed to be 14.86 per cent in the present study. The retrospective study in current study revealed the increased trends in mastitis prevalence over last ten years periods. This may be attributed to the emergence of resistant strains of bacteria due to indiscriminate use of antibiotics in mastitis. The present study found changing trends in mastitis over last ten years. In order to address twin challenges of major public health concern of antibiotic residues in milk and to minimal use of antibiotic to treat mastitis, study was conducted in 2 phases. in phase I animals affected with mastitis were screened and animals affected with mastitis were given oral citrate therapy to observe any deleterious effect of citrate on animal metabolism and standard dose of treatment was established. in phase II animals were divided into 3 groups animals in group I received only antibiotic therapy, group II received citrate therapy, group III citrate and antibiotic From present study it was found that oral administration of citrate @80 mg/kg b.wt has no deleterious effect on the metabolic profile of cows and was found to have potentiating effect on clinical recovery from mastitis. So in is concluded in present study that prevalence of mastitis is changing and Oral administration of citrate has a potential to replace or reduce the use of antibiotics in mastitis treatment to address the public health concern of antibiotic residues in milk and decreasing prevalence of mastitis.
The present study was aimed to develop and evaluate virulence marker Listeriolysin-O (LLO) peptide based ELISA for rapid and reliable serodiagnosis of Ovine listeriosis generally caused by two pathogenic strains of *Listeria* species namely *L. monocytogenes* (LM) and *L. ivanovii* (LI). A total of 200 clinical samples comprising of blood (30), vaginal swabs (47), faecal swabs (47), brain tissues of fetus (7), abomasal tissues and contents of fetus (10) and sera (59) were screened from organized sheep farms of Kashmir region. After standard isolation and biochemical confirmation the overall prevalence of *L. monocytogenes* was found to be 2.83%. Serologically, indirect plate ELISA revealed positivity for antibodies against listeriolysin O (ALLO) in 32(54.2%) out of 59 sera samples which after adsorption with streptolysin O (SLO) reduced to 14(23.7%), indicating the need for sera adsorption for removal of cross-reactivity. Two synthetic peptides (LLO-1 and LLO-2) representing major antigenic domains of Listeriolysin-O (LLO) were identified, analyzed, synthesized and were employed in indirect ELISA. On screening LLO-1 peptide an overall seropositivity of 18.6% was observed which after adsorption of sera with SLO reduced to 10.1%, whereas with regard to LLO-2 peptide the seropositivity was 15.2% before SLO adsorption which after adsorption was found to be 6.7%. Overall, result of seropositivity with LLO-1 and LLO-2 peptides revealed comparatively less cross-reactivity with that of native LLO.
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**TITLE**

**Novel Methods for an Effective Diagnostic Control of Leishmaniasis**

Leishmaniasis is a parasitic zoonotic disease caused by obligate intracellular protozoan parasites of genus *Leishmania* and belongs to family *trypanosomatidae*. These parasites develop different forms of the disease including cutaneous, mucosal and visceral leishmaniasis. The range of manifestation depends on immune level of the host, type of parasitic species involved and inflammatory reactions. Leishmaniasis causes high morbidity and mortality in underdeveloped and developed countries. In the current era, research has been focused on advanced techniques for rapid and effective diagnosis of these parasitic infestations. Effective laboratory techniques are mandatory for exact diagnosis of the disease particularly in the patients those are immuno-compromised due to HIV. The current review focus on the present state of diagnostic tools based on microscopic observations, detection of antigens, serological analysis including indirect fluorescent antibody test, ELISA, immunoblotting test, direct and latex agglutinations, immunochromatic test, polymerase chain reaction, loop mediated isothermal amplification test, intradermal leishmania skin test and nucleic acid sequence-based amplification test.
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**Title**

Acaricidal, anthelmintic and anti-protozoal activity of *Terminalia arjuna*, *Daphne mucronata* and *Chenopodium album*

Crude aqueous extracts (CAE) of three plants *Terminalia* (*T.* arjuna) (leaves), *Daphne* (*D.* mucronata) (leaves) and *Chenopodium* (*C.* album) (aerial parts) were evaluated for their acaricidal, anthelmintic and anticoccidial activities against *Rhipicephalus microplus*, *Haemocnthus contortus* and *Eimeria tenella* respectively. Plant extracts were prepared by separately boiling the chopped plant material in water. After filtration, 2-fold serial dilutions of *T. arjuna*, *D. mucronata* and *C. album* were prepared from their 2.8%, 4.5% and 6% concentrated (weight/volume) stock solutions, respectively. 1% stock solution of each extract was used to check the acaricidal activity through syringe test, anthelmintic activity through Adult Mortality Assay (AMA) and anti-coccidial activity by Sporulation Inhibition Assay (SIA). Results were subjected to probit analysis using software (PoloPlus) and one-way ANOVA followed by Tukey’s test to detect significance among the groups. In syringe test, non-significant difference (*P*>0.05) was found for LC$_{99}$ among all treatment groups. *C. album* extract was found with least value of LC$_{99}$=5.9% 24 hour post-treatment (PT). Results of 24 hours and six days PT indicated that CAE of plant material is slow acting. While LC$_{99}$ of *D. mucronata* was found to be 8.17% and for *T. arjuna*, LC$_{99}$ was 121.01%. In AMA, time dependent response was statistically significant (*P*<0.05) at different hours PT of stock concentrations for *C. album*. Similarly, other plants i.e. *T. arjuna* and *D. mucronata* showed non-significant results compared to levamisole @ 1.257% at 6 hour PT. For SIA, plant extracts were found with minimum LC$_{99}$ 3.1%, 6.2% and 19.4% for *T. arjuna*, *D. mucronata* and *C. album*, respectively.

**Keywords:** Plants, extract, LC$_{99}$
Phytochemicals, found in the plant materials, are considered to be relatively safer and environment friendly by the general public, as they have less side effects on human and animal health. Different combinations of crude aqueous extracts of three plants *Terminalia* (*T.* *arjuna*) (leaves), *Daphne* (*D.* *mucronata*) (leaves) and *Chenopodium* (*C.* *album*) (arial parts) were evaluated for their anthelmintic and anticoccidial activities against *Haemocnhus contortus* and *Eimeria tenella* respectively. 1% stock solution of each extract was used to check the anthelmintic activity through Adult Mortality Assay (AMA) and anti-coccidial activity by Sporulation Inhibition Assay (SIA). Results were subjected to probit analysis using software (PoloPlus) and one-way ANOVA followed by Tukey’s test to detect significance among the groups. In AMA, time dependent response was statistically significant (*P*<0.05) at different hours post treatment (PT). Stock concentrations of combinations of *C. album* + *D. mucronata* and *T. arjuna* + *C. album* were found comparatively non-significant at 4 hour PT with positive control group i.e., levamisole @ 1.257%. Similarly, other combination of *C. album* + *D. mucronata* + *T. arjuna* showed non-significant results compared to levamisole @ 1.257% at 6 hour PT. In SIA, comparison of LC$_{50}$, LC$_{90}$ and LC$_{99}$ with 95% CI indicated significant differences for all treatment groups. LC$_{90}$ and LC$_{99}$ values were 18.83% and 582% for *T. arjuna* + *D. mucronata*, with non-significant difference among all other treatments. There was an increasing trend of sporulation inhibition with an increase in the dose percentage.

**Keywords:** Plant extracts, Adult mortality assay, Sporulation inhibition assay
Toxocariasis is an important parasitic disease of dogs and cats having great zoonotic importance. Toxocara canis and Toxocara cati are the main causative agents of this disease in dogs and cats respectively. However, prevalence of *Toxocara canis* is high due to increased host to host transmission, therefore, easily acquired by human. The incidence of *T. canis* is low due to minor larval viability in paratenic host. Ocular and visceral larval migrans are the main outcomes of this disease in peoples. Pathogen spreads in adult by ingestion of undercooked meat containing Toxocara larva. In most cases, clinical signs and symptoms are not shown by this problem. Sometime, this infection remain undiagnosed and the reason behind this expensive or unavailability of diagnostic test. Molecular and serological assays are most important diagnostic test. Anthelmintics such as albendazole and febendazole are the good hope in the face of above discussed challenges in controlling toxocariasis because of having good properties against toxocariasis. The aim of this article is to provide a comprehensive account of knowledge about toxocariasis such as its mode of transmission, diagnostic tools, treatment, control strategies including public health importance.
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A case study causes deteriorating health of Schizothorax---an important food fish of Kashmir

The food we eat influence our health and fish is one of the important and healthy source of food. The food besides containing protein and other nutrients such as vitamin D, it contains a specific type of fats i.e. omega-3 fatty acids, that may reduce the risk of developing heart disease and other medical problems. In Jammu and Kashmir fisheries has the potential to grow exponentially in view of its unique agro-climatic conditions and abundant water bodies in the form of lakes and rivers. But Census reports reveal that there is a constant decrease in the fish production which is attributed to a number of factors especially parasitism and pollution; which warrants a thorough study of these factors especially parasitic infestation. In order to understand level of damage caused by parasitism and pollution, parasitological investigation was carried out in the Schizothorax niger (Heckel 1838) for a period of one year in River Sindh. A strong positive correlation (Pearson’s correlation, r=0.9; P=0.038) between total length of Schizothorax and number of Helminths was observed. Prevalence and mean abundance were positively and significantly correlated with season (r=0.7, P<0.01 and r=0.9, P<0.01, respectively). Thus seasonal dynamics, total length and weight of the host significantly influenced the Helminth infection. The above findings will be useful in devising the appropriate control strategies for the Helminth in wild fish in Kashmir valley as well as in similar climatic zones of other parts of the world. Also, information from this study will be used to assess the spread and extent of the Helminth infection which is a potential threat to the indigenous fish fauna of river Sindh.
Toxoplasmosis is a common zoonotic problem caused by a protozoon parasite; *Toxoplasma (T.) gondii* (Eucoccidiorida: Sarcocystidae) in animals and humans. Heteroxenous facultative life cycle of this parasites is responsible for development of multiple tissue cyst in muscular structure particularly in cardiac, skeletal and nervous tissues of the food animals which may remain infectious for years. The global burden of toxoplasmosis in livestock and human population has been documented as 10-92%. In Pakistan, distribution of toxoplasmosis in livestock population is reported ranging from 14 to 37%. Screening of domestic livestock species for prevalence of toxoplasmosis and to understand their role in spread of human infection is very much necessary. For this purpose, rapid and accurate diagnostic methods can play significant role for management of toxoplasmosis. Etiological, imaging and immunological techniques are regarded as conventional approaches for the diagnosis of toxoplasmosis. Indirect haemagglutination test, indirect immunofluorescence, enzyme linked immunosorbsent assay (ELISA) and Latex Agglutination Test (LAT) are most widely used conventional serological assays worldwide. Genetic characterization of *T. gondii* may be carried out through Western blotting, PCR, RT-PCR, DNA sequencing. While non-DNA-based diagnostic methods include serotyping techniques. Use of synthetic polymorphic peptides derived from *T. gondii* antigens is a simple, cost effective, sensitive and specific typing method for type specific toxoplasmosis in human and animals. The purpose of this presentation is to disseminate knowledge regarding available techniques and sensitivity for accurate diagnosis of toxoplasmosis in human and animals. These advances will contribute to an improved understanding of the epidemiology, prevention and control of toxoplasmosis.
Abomasum is the predilection site for living bursate nematodes belonging to family Trichstrongylidae. Among pathogenic species of gastrointestinal (GI) nematodes, *Haemonchus (H.) contortus* is highly pathogenic and economically important nematode parasite of goats round the globe. Present study was planned to investigate the comparative resistance of ingenious breeds of goat towards natural infection of *H. contortus*. To this end, abomasa were collected on monthly bases from goat slaughtered at abattoir at metropolitan Faisalabad. Collected samples were than brought to Molecular Parasitology Laboratory, Department of Parasitology, University of Agriculture, Faisalabad for processing and examination. All the abomasa were processed for worm recovery, identification and total worm count. Further, gravid female worms were processed for uterine egg count through modified McMaster egg counting technique for each breed of goat. Over all occurrence of *H. contortus* was significantly higher (66.67%) in Beetal goats as compared to Teddy goats (42.50%). Breed and age were found statistically associated (*P* < 0.05) with the prevalence of haemonchosis while no statistical association (*P* > 0.05) was found in case of gender of the goats. There was significantly lower (*P*<0.05) total worm burden, uterine egg count of gravid females, male/female ratio in Teddy as compared with Beetle goat breeds. Present study has provided the data on the resistant goat breeds in study area. This indication of breed resistance towards *H. contortus* will provide the guidelines in selective breeding of disease resistant livestock in Pakistan, which is an indirect way to control the parasite related losses.
Fish is a food of excellent nutritional value, providing high quality protein and a wide variety of vitamins and minerals to humans. During its aquatic life, fish can be exposed to numerous parasites that may cause serious illness in their consumers. Globally, over 50 species of helminth parasites from fishes, crabs, crayfishes, snails, and bivalves are known to produce human infections. The most important parasites acquired by the human from fish are belong to nematodes (*Anisakis simplex* and *pseudoterranova decipiens*), castodes (*Diphyllobothrium*), trematodes (family heterophyidae, Opisthorchiidae and Nanophyetidae), and acanthocephalans. The infections associated with acanthocephalans are rarely reported in humans. Globally, 20 million people estimated to be infected by the fish borne parasites and almost 20,000 cases are contributed to *Anisakis simplex*; a major fish borne zoonotic parasite. Some cultural, social and behavioral practices such as consumption of raw fish, are the major risk factor for disease incidence. Upon consumption, these parasites may lead to sever abdominal discomforts such as diarrhea, cramps, ulcers, nausea, vomiting and associated allergies, weakness and many other symptoms. The methods of capture, handling and storage directly affect the quality of sea food associated with the presence of parasites. That’s why to reduce the risk of zoonosis; measures can be taken during harvesting, processing and post processing. Most of seafood zoonoses occur along coastal regions where seafood products are commonly consumed. The purpose of this presentation is to disseminate awareness to public and to motivate scientific community to screen the fish of Pakistan for distribution of parasite so that appropriate prophylactic measures may be devised.
Ticks are by far the best transmitters of fatal pathogens to livestock and humans. Powassan virus (POWV) is an arthropod-borne virus (arbovirus) in the family of Flaviviridae, genus Flavivirus. Human POWV infections are rare but cause significant disease. Symptoms include acute onset of fever, weakness, confusion, headache, nausea, vomiting, and stiff neck. Severe signs and symptoms include respiratory distress, tremors, seizures, paralysis, and coma. Most individuals with POWV infections develop meningoencephalitis and many have long-term neurologic sequelae; 10%-15% of cases are fatal. POWV is transmitted between small and medium-sized mammals by ixodid ticks. It is transmitted to humans through the bite of an infected tick. Since the late 1990, the incidence of human disease seems to be increasing. In endemic areas, up to 7% ixodid ticks may carry POWV and a high seroprevalance (up to 90%) has been reported in small mammalian host from these regions. Globally, POWV has been isolated from several North American tick species, including Ixodes spp. (I. cookei, I. marxi, and I. spinipalpus) and Dermacentor andersoni. POWV is a member of Tick-borne encephalitis complex of viruses. These viruses are known to be major health issue in Asia and Europe with estimated case reports of 10,000-15,000 annually. Diagnosis of POWV is usually carried out through an IgM antibody capture ELISA. The best way to prevent POWV is to decrease potential exposure to ticks. In Pakistan, the status of POWV is still unknown. In this scenario, this region may be screened for presence of POWV and measures should be taken to minimize the exposure of humans to ticks.
Protozoa of the genus *Cryptosporidium* are described as important gastrointestinal pathogens of different hosts, including livestock, companion animals and wild animals. Within this epidemiological chain, some species of this genus are pathogenic for humans. Cryptosporidiosis is characterized by acute gastro-intestinal disturbances, hemorrhagic or mucoid diarrhoea, lethargy, fever, anorexia and loss of condition leading to significant economic loss in farm animals, especially young or neonates. In the present study, examination of 200 fecal samples of cattle calves by modified Ziehl-Neelsen stain revealed presence of *Cryptosporidium* oocysts in 48.5% (97/200) pre-weaned cattle calves of Jammu and Kashmir. Highest prevalence was recorded in calves of age group 16-30 days (62%), followed by < 15 days of age group (54%), 31-45 days of age group (46%) and 46-90 days of age group (32%). The prevalence of *Cryptosporidium* spp. infection was significantly higher in diarrhoeic calves (59%) than non-diarrhoeic calves (38%), irrespective of age, season and sex. Male calves showed a higher incidence of infection (68.29% diarrhoeic calves; 52.17% non-diarrhoeic calves) as compared to female calves (52.54% diarrhoeic calves; 33.76% non-diarrhoeic calves). As per season, prevalence was highest in winter (68%), followed by monsoon (54%), post-monsoon (46%) and summer (26%). Cattle calves having mucus in the faeces showed significantly higher prevalence (81.15%) of *Cryptosporidium* infection than those having blood in the faeces (9.67%). Genetic characterisation of 40 samples found positive by modified Ziehl-Neelson staining was carried using molecular techniques. Nested PCR of 18S small subunit (SSU) rRNA gene of *Cryptosporidium* species amplified a product of 830 bp. RFLP analysis of nested PCR product by three restriction enzymes viz., *SspI*, *VspI* and *MboII* was carried and only one species namely *Cryptosporidium parvum* was found to infect pre-weaned cattle calves.

**Keywords:** Cryptosporidiosis, Pre-weaned cattle calves, Molecular characterization.
Toxoplasmosis is the most common zoonotic disease which is caused by a protozoan parasite *Toxoplasma gondii*. It is an obligate intracellular Apicomplexan parasite which infects humans and a wide range of warm-blooded animals. Cancer remains a leading cause of death worldwide. The prevalence of *T. gondii* is much higher in cancer patients but its prevalence also common in other patients like those with neoplasia, haemodialysis, in patients with renal transplants. The prevalence of toxoplasmosis is also higher in immunocompromised patients. The objective of this study was mainly the serodiagnosis of *T. gondii* in cancer patients. *T. gondii* was diagnosed in the serum of the subjects by using commercially available ELISA kit. The data regarding the sex, age, weight, socioeconomic status, educational level, and occupation, type of cancer and presence or absence of dog or cat as pet animal was also recorded with the help of a specially designed questionnaire. Overall prevalence of toxoplasmosis in cancer patients was found 53.4%. Maximum 85% seroprevalence was noted in ovarian cancer patients. *T. gondii* infection is a serious and severe problem in cancer patients and in this study an attempt was made to identify the risk factor. Some precautionary measures were suggested to prevent to prevent or at least to control toxoplasmosis in cancer patients.

**Keywords:** *Toxoplasma gondii*, Cancer patients, haemodialysis, neoplasia, IgG ,ELISA
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**TITLE**

Distribution of Buffalo Tick Species in Sheikhupura District

The ticks were collected from naturally infested 120 buffalo in district Sheikhupura. All the ticks were identified in Entomology laboratory of UVAS, Lahore by using tick identification key of A.R. Walker 2014 (ISBN 0-9545173-0-X). From these 120 animals 453 ticks were collected. 252 (55.62%) were *Hyalomma* and 201 (44.37%) were *Boophilus*. Among *Hyalomma* the distribution pattern of different species was identified as; *H. dromedrii* (20.23%), *H. anatolicum excavatum* (15.47%), *H. marginatum marginatum* (14.28%), *H. impressum* (11.90%), *H. anatolicum anatolicum* (9.52%), *H. detritum detritum* (9.52%), *H. impeltatum* (7.14%), *H. detritum scupense* (4.76%), *H. marginatum rufipes* (3.75%), *H. truncatum* (2.3%), *H. marginatum turanicum* (1.19%). While in *Hyalomma* males were 42.85% and females were 57.14%. Similarly a total of 201 ticks were identified as *Boophilus* among which the distribution pattern of different species was identified as; *B. microplus* (43.28%), *B. annualtus* (35.82%), *B. decoloratus* (13.4%), *B. geigyi* (7.4%). While in *Boophilus*, 20.89% were male and 79.10% were females. Here we can conclude that *H. dromedrii* and *B. microplus* is more distributed in buffalo of Sheikhupura district.

**Keywords:** A.R. walker key, *B. microplus*, cattle, *H. dromedrii*, Ticks.
The ticks were collected from naturally infested 120 cattle in district Sheikhupura. All the ticks were identified in Entomology laboratory of UVAS, Lahore by using tick identification key of A.R. Walker 2014 (ISBN 0-9545173-0-X). From these 120 animals 666 ticks were collected. 240 (36%) were *Hyalomma* and 426 (69%) were *Boophilus*. Among *Hyalomma* the distribution pattern of different species was identified as; *H. anatolicum excavatum* (23.75%), *H. dromedrii* (18.75%), *H. anatolicum anatolicum* (17.5%), *H. impeltatum* (8.75%), *H. detritum scupense* (8.75%), *H. impressum* (6.25%), *H. marginatum marginatum* (6.25%), *H. marginatum rufipes* (5%), *H. detritum detritum* (5%). While in *Hyalomma* males were 31.25% and females were 68.75%. Similarly a total of 426 ticks were identified as *Boophilus* among which the distribution pattern of different species was identified as; *B. annulatus* (37.32%), *B. decoloratus* (31.61), *B. microplus* (21.12%), *B. geigyi* (9.85%). While in *Boophilus* 5.65% were male and 94% were females. Here we can conclude that *H. anatolicum excavatum* and *B. annulatus* is more distributed in cattle of Sheikhupura district.

**Keywords:** A.R. walker key, *B. annulatus*, cattle, *H. anatolicum excavatum*, Ticks.
Toxoplasmosis is caused by *Toxoplasma gondii*, which is an obligate intracellular apicomplexan protozoan parasite. The infection is highly prevalent worldwide, and is important both from veterinary and human health concern. In the past recombinant surface antigen-1 (rSAG-1) has been shown to be a good candidate for the development of immuno-diagnostic kits as well as Vaccine. Through immuno-blotting, Surface antigen-1 has been identified by sera collected from *Toxoplasma gondii* infected cats, dogs and humans. Looking at the importance of this health threatening issue, the current study was designed to characterize rSAG-1 through western blotting for development of local diagnostic kit through western blotting using mouse anti-serum. Recombinant SAG-1 was quantified by using commercial kit based on BCA assay. The 15 µg of rSAG-1 was inoculated subcutaneously (S/C) 3 times with each 2 weeks interval in mice to raise hyper-immune serum. Blood was collected from mice two weeks after the each injection through lateral Retro-Orbital Bleeding. Serum was collected by centrifugation. The rSAG-1 was electrophoresed on 12% polyacrylamide gel through SDS-PAGE technique and the protein was transferred to nitrocellulose membrane for western blotting. Anti-serum raised against rSAG-1 was cross-reacted with the rSAG-1 already immobilized on the nitrocellulose membrane. Anti-mice immunoglobulin G conjugated with Alkaline Phosphatase (AP) was used as secondary antibodies for the development of immuno-blot. Immuno-blot revealed a band of 35 KDa.
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### TITLE

Biochemical Resistance Study of *Anopheles subpictus* in District Kasur Pakistan

*Anopheles subpictus* Grassi (Diptera: Culicidae) is a malaria vector in South Asia, where insecticides mainly pyrethroids are used for vector control interventions to minimize the malaria burden. However, subsequent resistance due to detoxifying enzymes in mosquito population towards insecticides has been ascribed to indiscriminate use of chemicals. Information on elevated detoxifying enzymes in mosquitoes is helpful for adapting alternative strategies in vector control interventions. The aim of the study was to detect phenotypic resistance against DDT, deltamethrin and permethrin. Activities of detoxifying enzymes, i.e. esterases, Glutathion S-Transferases (GST), monooxygenases (MFO) and insensitivity of acetylcholinesterases (AChE) were evaluated in susceptible and field strains of *An. subpictus*. Field collected adult blood fed females of *An. subpictus* was reared in the insectary for F1 generations which were evaluated by using WHO susceptibility bioassays. Variations in the activity of detoxifying enzymes were detected, using standard biochemical assays, in *An. subpictus*. Adult mosquitoes had shown resistance against DDT, deltamethrin and permethrin. Percentage mortalities recorded were DDT 25.01 %, deltamethrin 54.73 % and permethrin 74.22 %. Results revealed significantly ($P< 0.0001$) elevated levels of detoxifying enzyme i.e. $57.41 \pm 8.073$ AChE, $0.469 \pm 0.115$ GST, $11.665 \pm 4.165$ MFO, $3.968 \pm 2.311$ and $1.551 \pm 0.7610$ NSE mmol/min/mg protein activities in resistant strain when compared with susceptible strains of *An. subpictus*. The existence of a metabolic resistance mechanism intimidates sustainability of mosquito controlling interventions in this area. The study suggests alternative, cost effective measures should be necessitated along-with continuous monitoring of resistance surveillance of malaria vectors in endemic regions.

**Keywords:** *Anopheles subpictus*, Biochemical assay, Insecticide resistance, Public health
Efficacy Of Ginger against Gastro-Intestinal Tract Nematodes in Cattle in District Rajanpur

Nematodes infestation is one of the major problems of animals in Pakistan, causing huge economic losses in bovines. These are one of the major threats to livestock productivity. The anthelmintic effect of dried powder of ginger was evaluated against the gastrointestinal nematodes of cattle in District Rajanpur. A total 150 cattle positive for GIT nematodes were included in this study. These cattle randomly divided into 5 groups of 30 viz A, B, C, D and E, treated with Ginger @ 3 g/kg, 3.5 g/kg, 4 g/kg and Gurh (placebo) @ 1 kg by per oral route, respectively. Fecal sample from each of cattle in all groups were collected directly from rectum at day 0 (pre-treatment) and then at day 14 (post-treatment) for EPG calculation by McMaster technique. The efficacy of ginger was calculated by using The Fecal Egg Count Reduction Test (FECRT). Anthelmintic efficacy of Group B, Group C, Group D and Group E were 86.20%, 91.39%, 89.65%, 87.05%. Ginger at dose rate of 3.5 g/kg, showed maximum EPG reduction. The current study highlights the effect of ginger against worms of cattle, and hence is a potential alternate for different allopathic medicines against which resistance has developed or is developing.
The aim of this study was to find out the antiviral and embryonic toxicity of ivermectin and ibuprofen alone and in combination against H9 virus by using embryonated chicken eggs model. Different concentrations of ivermectin (100, 50 and 25 µg/ml), Ibuprofen (50, 25 and 12.5 µg/ml) and amantadine (1000, 500 and 250 µg/ml) were used. Two fold dilutions, mixed with avian influenza H9 virus were inoculated in embryonated chicken eggs. Haemagglutination test was performed for evaluation of antiviral activity. For embryo toxicity, dilutions were made in phosphate buffer saline without virus and mortality ratio was checked after incubation. Strong antiviral activity was exhibited by ivermectin at higher concentration but same concentration was embryotoxic however lower concentrations were devoid of toxicity with moderate antiviral activity. In case of ibuprofen weak antiviral activity was exhibited, however all concentrations were devoid of toxicity. When drugs were administered in combination strong antiviral activity was exhibited at all concentrations and these were free of toxicity.

**Keywords:** Ivermectin, Ibuprofen, Avian Influenza H9, Haemagglutination.
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The ticks were collected from naturally infested 120 goats in district Sheikhupura. All the ticks were identified in Entomology Laboratory of UVAS, Lahore by using tick identification key of A.R. Walker 2014 (ISBN 0-9545173-0-X). From these 120 animals 252 ticks were collected. 228 (90%) were Rhipicephalus and 24 (10%) were Hyalomma. Among Rhipicephalus the distribution pattern of different species was identified as; R. appendiculatus (42%), R. senegalensis (23.68%), R. mubsamae (10.51%), R. guilboni (7%), R. camicasi (4.38%), R. zambenziensis (3.5%), R. simus (3.5%), R. sanguineus (2.6%), R. parvus (1.7%), R. paratextatus (0.87%). While in Rhipicephalus males were 37.7% and females were 62%. Similarly a total of 24 ticks were identified as Hyalomma among which the distribution pattern of different species was identified as; H. dromedrii (25%), H. marginatum turanicum (25%), H. detritum detritum (16.6%), H. anatolicum excavatum (8.3%), H. impeltatum (8.3%), H. impressum (8.3%), H. albipamatum (8.3%). While in Hyalomma 58% were male and 42% were females. Here we can conclude that R. appendiculatus is more distributed and Hyalomma species are less common in goats of Sheikhupura district.

Keywords: A.R. walker key, goat, R. appendiculatus, Ticks.
Caprines reared at Mountain Research Center for Sheep and Goat (MRCSG), Srinagar, Kashmir exhibited reduced efficacy to closantel and ivermectin (IVM) in Faecal Egg Count Reduction Test (FECRT). The results suggested that the overall efficacy for IVM was 83.54% and 90.00% on 7th and 14th day post-treatment, respectively and for closantel it was 68.35% and 86.25% on 7th and 14th day post-treatment, respectively. The pre-treatment faecal culture revealed *Haemonchus contortus*, *Ostertagia circumcincta* and *Trichostrongylus colubriformis* as predominant strongyles, however, in post-treatment samples, *H. contortus* was only observed. The survey indicated that the Gastro-intestinal nematodes (GINs) especially *H. contortus* of goats on MRCSG have developed multiple anthelmintic resistance to closantel and IVM and the condition is alarming on the farm.

**Keywords:** Closantel. FECRT. GI Nematodes. Goats. IVM.
Goats reared at Mountain Research Center for Sheep and Goat, SKUAST-K, exhibited reduced efficacy to treatment with fenbendazole (FBZ) in Faecal Egg Count Reduction Test (FECRT). The overall efficacy for FBZ on 7th and 14th day post-treatment was 44.30% and 62.50% respectively. The pre-treatment faecal culture revealed *Haemonchus contortus*, *Ostertagia circumcincta* and *Trichostrongylus colubriformis* as predominant strongyles, however, in post-treatment samples, *H. contortus* was only observed. Further, the infective larvae were subjected to Allele specific PCR (AS-PCR) for accurate diagnosis of BZ resistance. The AS-PCR revealed 52% of *H. contortus* were homozygous resistant (rr) and 17% were heterozygous (rS) on day “0” before treatment and 100% homozygous resistant (rr) on 7th day post treatment. In both *T. colubriformis* and *O. circumcincta*, 100% population was homozygous susceptible (SS) at day “0” before treatment. The overall frequency of resistant (r) allele for *H. contortus* was 58% and for susceptible allele (s) was 42%. For *T. colubriformis* and *O. circumcincta* the frequency of susceptible allele (s) was 12% and 7% respectively. The survey indicated that the Gastro-intestinal nematodes (GINs) of goats on MRCSG have developed BZ resistance and the condition is alarming at the farm.

**Keywords:** AS-PCR. FBZ. FECRT. GI Nematodes. Goats.
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TITLE

Epidemiology and Therapeutic Study on Gastrointestinal Nematodosis in Sheep

A survey was conducted on the prevalence of gastrointestinal helminth parasites in sheep reared in organized and unorganized areas of Srinagar and Ganderbal districts of Kashmir valley (India) for a period of nearly one year from September 2010 to July 2011. A total of 1200 fecal samples were examined of which 925 (77.08%) were found positive for one or other gastrointestinal nematode parasite and prevalence was higher in unorganized farms (80.00%) as compared to organized farms (73.33%). Season wise prevalence during summer, spring, winter and autumn was 83.00, 81.00, 73.00 and 71.33% respectively, being highest in summer and lowest in autumn. Prevalence of 84.88% was recorded in adult (6-12 months) and 72.72% in young sheep (1-6 months). Sex-wise the prevalence in males was 78.60% and in female sheep was 76.00%. Based on coporoculture, the prevalence of different genera of GIN parasites was: *Haemonchus* spp. (59.75%), *Trichostrongylus* spp. (36.91%), *Ostertagia* spp. (36.66%), *Nematodirus* spp. (19.33%), *Bunostomum* spp. (17.08%), *Oesophagostomum* spp. (15.66%), *Trichuris* spp. (12.66%) and *Marshallagia* spp. (6.16%). In infected animals hematobiochemical studies revealed significant decrease in hemoglobin, packed cell volume, lymphocyte, neutrophil and total erythrocyte count and increase in eosinophil and monocyte percentage. Serum glucose, protein and albumin values were significantly decreased. The fecal egg count reduction test (FECRT) showed highest efficacy against ivermectin (91.93%) followed by fenbendazole (89.09%), artemesia (69.23%) and closantel (59.25%).

85
Opportunistic pathogens represent the type of pathogens which infect only those individuals with impaired immune system and lead to disease that can be severe and difficult to treat the immunocompromized host. This study was designed to identify the opportunistic bacterial pathogens i.e. *Pseudomonas* and *Citrobacter* infections in immunocompromized chronic liver disease (CLD) patients by analyzing sensitivity pattern of bacterial pathogens against commonly used antibiotics like quinolones, aminoglycosides, cephalosporin and co-trimoxazole. Blood and urine samples were collected from infected CLD patients with suspected signs and symptoms by using aseptic measures. The pathogens were isolated, identified and purified by selective culturing methods, which were subjected to active growth to check the sensitivity against subjected antibiotics by Kirby Bauer disc diffusion method. *Pseudomonas* and *Citrobacter* pathogens proved to be a multi-resistant pathogens and use of combination of antibiotics proved to be more effective. The chances of recurrent infection among the immunocompromized patients were minimized. The effect of different combination of antibiotics in CLD patients showed that combination of ceftriaxone and amikacin prove to be more effective during clinical use. However, in-vitro studies revealed that combination of cephradine with gentamycin had high susceptibility (97%). It was concluded that combine therapy of different antibiotics could be more useful in CLD patients.

**Keywords:** Quinolones, Amino Glycosides, Cephalosporin, Co-trimoxazole
### Title

Evaluation of The Diagnostic Potential of Exosomes-Like Extracellular Vesicle Proteins for Schistosomiasis Japonica

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Schistosomiasis is a chronic parasitic disease caused by the genus Schistosoma and pose great threat to human and animal health. Accurate diagnosis and treatment are critical to reducing the rates of morbidity and mortality. The diagnostic standard for active schistosomiasis is viable eggs in faeces (S. japonicum, S. mansoni), or tissue biopsies. However, the presence of infecting schistosomes cannot be ruled out definitively because of the low sensitivity of standard urine and faecal examinations. Consequently, there is a great need for sensitive and non-invasive biomarkers for precise diagnosis of this disease. Previously, we identified a total of 403 proteins in S. japonicum exosomes-like extracellular vesicles. Here, we further analyzed these proteins by bioinformatics and found four schistosome specific proteins with low homolog with mammalian hosts. Then, we evaluated the diagnostic potential of these four proteins for schistosomiasis by ELISA. Our preliminary results suggested that these proteins could serve as potential non-invasive biomarkers for schistosomiasis.
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With the passage of each day, the parasites are becoming more and more drug resistant thus reducing the options for their treatment. There is a need to explore herbal drugs having anthelmintic activity. The aim of this study was to evaluate therapeutic efficacy of \textit{Euphorbia} plant against gastrointestinal nematodes of goats. \textit{Euphorbia} plant extracts have efficacy against GIT nematodes in goats. Eighty goats were selected for study. Goats were divided into 8 groups (Group A (control), B, C and D), and each group containing 10 goats. Further group B (treated with crude powder) and group C (treated with methanol extract) were divided in to sub-groups for dose evaluation of \textit{Euphorbia} plant extracts. Animals in group D were drenched with albendazole @ 3.8 mg/ kg. The result reveals that methanol extract 53.96 \% were more efficient ($P<0.05$) as compared to powder farm 23.14 \%. Animals were treated on day zero with (CME) and (CP) of \textit{Euphorbia} plant and albendazole. Sampling was done on day 0 pre-treatment 7, 14 and 21 post-treatment for eggs per gram of faeces (EPG) calculations. Data was tabulated and analyzed through one way ANOVA, $P<0.05$ were considered significant differences.