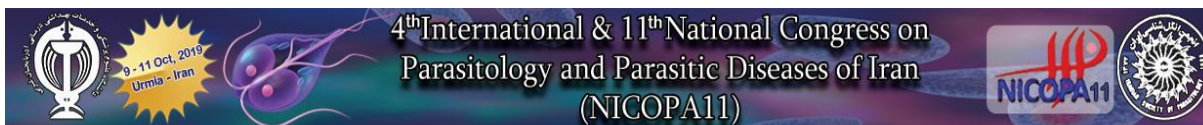




Abstracts

**4th International & 11th National Congress of Parasitology
& Parasitic Diseases of Iran (NICOPA11)**

**11-13 October 2019
Urmia-Iran**



4th International Congress and the 11th National Congress of Parasitology and Parasitic Diseases of Iran (NICOPA11)

9-11 Oct, 2019

Urmia-Iran

Organizer:

Urmia University of Medical Sciences

Collaborator:

Iranian Society of Parasitology

Congress President:

Dr. Javad Aghazadeh

Congress Scientific Secretary:

Dr. Khosrow Hazrati Tappeh

Deputy of Chair-Scientific Committee:

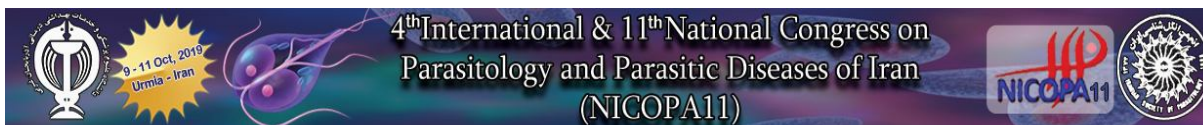
Dr. Shahram Khademvatan

Congress Executive Secretary:

Dr. Iraj Mohebbi

Deputy of Chair-Executive Committee:

Dr. Habib Mohammadzadeh



In the name of God

I would like to extend my sincere thanks to Almighty God who helped Urmia University of Medical Sciences (UMSU) and Iranian Scientific Association of Parasitology in conducting the Fourth International Congress and the Eleventh National Congress of Parasitology in Urmia. In spite of many difficulties and obstacles in the development of medical sciences in our country, the Iranian medical community has demonstrated praiseworthy endeavors in providing medical services to patients. Due to the urgent need for developing the level of knowledge in medical sciences, UMSU is honored to host prominent scholars and scientists in this Congress. The most sincere efforts of the respected professors for advancing the goals of the Congress are really admirable. It is hoped that in your presence, the transfer of the experience and research carried out in recent years would lead to the promotion of scientific knowledge and skills in the field of parasitology, and pave the way for improving the quality of services.

Dr. Javad Aghazadeh

Chancellor of Urmia University of Medical Sciences

President of the Congress



In the name of God

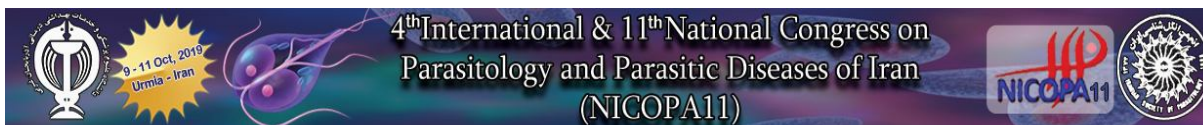
Congresses make the opportunity to pose scientific subject matters and disputable topics in the form of lectures, collaborations, and deliberations in the presence of the experienced professor. Meanwhile, the young colleagues will be able to add to their knowledge and experiences through these subjects and gatherings. In this international Congress that will be held in Urmia, the efforts of domestic and foreign parasitologists will be presented in special sections and panels. It is hoped that your presence from all over the world and from Iran will inspire and contribute to this professional team.

Dr. Khsorow Hazrati Tapeh
Scientific chairman of the Congress

کمیته علمی چهارمین کنگره بین المللی و یازدهمین کنگره ملی انگل شناسی و بیماری های انگلی ایران
۱۹ - ۱۷ مهر ماه ۱۳۹۸ - ارومیه

ردیف	نام خانوادگی	نام	مدرک تحصیلی	رشته تخصصی	مرتبه علمی	نام مرکز
۱	دکتر آقازاده	جواد	دکترای تخصصی	جراحی مغز و اعصاب	استاد	رئیس دانشگاه علوم پزشکی ارومیه
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۳	دکتر ادریسیان	غلامحسین	دکتری تخصصی	انگل شناسی پزشکی	استاد	دانشگاه علوم پزشکی تهران
۴	دکتر اورمزدی	هرمزد	دکتری تخصصی	انگل شناسی پزشکی	استاد	دانشگاه علوم پزشکی ایران
۵	دکتر حقوقی راد	ناصر	دکتری تخصصی	انگل شناسی پزشکی	استاد	دانشگاه آزاد اسلامی
۶	دکتر حقیقی	علی	دکتری تخصصی	انگل شناسی پزشکی	استاد	دانشگاه علوم پزشکی شهید بهشتی
۷	دکتر خامسی پور	علی	دکتری تخصصی	انگل شناسی پزشکی	استاد	دانشگاه علوم پزشکی تهران
۸	دکتر دلیمی اصل	عبدالحسین	دکتری تخصصی	انگل شناسی پزشکی	استاد	دانشگاه تربیت مدرس
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۱۰	دکتر رکنی	محمد باقر	دکتری تخصصی	انگل شناسی پزشکی	استاد	دانشگاه علوم پزشکی تهران
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The 11th National and the 4th International Congress of Parasitology and Parasitic Diseases

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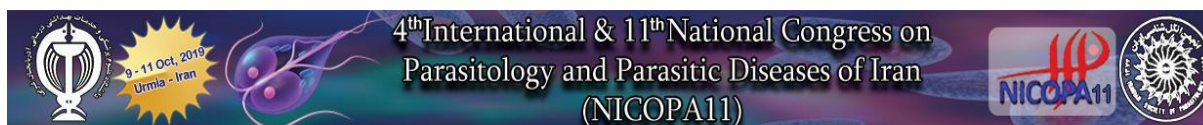
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Oral

Asymptomatic Human Blood Donor's Carriers of *Leishmania infantum*: Potential Reservoirs for Visceral Leishmaniasis in Northwestern Iran

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Aim: Little is known regarding transfusion-transmitted leishmaniasis (TTL) and the real global incidence of visceral leishmaniasis (VL) among blood donors as carriers of *Leishmania spp.* in endemic VL foci including Iran. Recent epidemiological evidences indicate that blood donor may be harbor of *Leishmania infantum* (*L. infantum*) infection in Iran.

Methods: The aim of the present study was to determine the prevalence of *Leishmania* infection among blood donors in a main endemic focus of VL in Iran using DAT and polymerase chain reaction (PCR) based methods. Between July–September 2016, blood samples were collected from 600 apparently healthy blood donors from six blood donation centers and blood donation mobile unit in Ardabil Province, where VL cases had been recorded. Each of these samples was tested for anti-*Leishmania* antibodies, in direct agglutination test (DAT), and for *L. infantum* kDNA, the PCR-based assay.

Results: Of 600 blood donors, which were examined, 23 (3.8%) blood donors were seropositive by DAT and of 23 seropositive subjects, 82.6% (19/23) were positive by PCR. All the seropositive cases were male except one of them was female.

Conclusion: Our findings showed that many asymptomatic human carriers of *L. infantum* live in the endemic regions of northwestern Iran and potentially act as reservoirs of infection; those must be considered carefully by arrangement VL control strategies in the country.

Keywords: Visceral leishmaniasis, *Leishmania infantum*, Blood donor, DAT, PCR.

Identification and Assessment on Excreted-Secreted/Somatic Immunoreactive proteins gained from adult *Dirofilaria immitis* using FPLC and MALDI-TOF mass spectrometry techniques

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Dirofilaria immitis is a roundworms and cause dirofilariasis in animals and rarely humans. Dirofilariasis is now recognized as one of the main world problems in small animal medicine. This study describes findings on the E/S proteins released during in vitro cultivation and somatic proteins of adult worms. The objective of the present study was to identify of the knowledge about *D. immitis* adult worms immunoreactive proteins using immunoblotting, mass spectrometry (MALDI-TOF) and fast protein liquid chromatography (FPLC) technique. Results showed that, the most immunoreactive proteins are at an range of 10-48 and 75 kDa and the six main sheared proteins between E/S and somatic compounds were including; Polyprotein antigen, P22u, Pepsin inhibitor Dit33, Neutrophil chemotactic factor DiNCF precursor, HSP 70. FPLC *D. immitis* antigen fractions were isolated at two peaks and immunoreactive bands were identified. Use of identified the immunoreactive proteins in the present study may provide new horizons into effective models to develop these diagnostic assays. Development of quantitative and practical parasite-specific assays to assess the level of infection for filarial nematodes.

Keywords: *Dirofilaria immitis*; dirofilariasis; Dog; Excretory-Secretory; Somatic; Immunoreactive protein; MALDI-TOF; FPLC

Evaluating the effectiveness of alum-propranolol mixture, as an adjuvant, on the immunogenicity of excreted/secreted antigens of *Toxoplasma gondii* RH strain in BALB/c mice

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Background: Adjuvants have been developed to raise the efficacy profile of target antigen-induced immune responses. Evolution of vaccines for *Toxoplasma* infection has taken longer after decades of research. The introduction of novel adjuvants is an important step in attempts to develop a safe and more efficient vaccine for this global threat.

Objectives: The present study was performed to determine whether the use of a mixed beta-adrenergic receptor antagonist, propranolol (PRP) and aluminum salt (alum) as an adjuvant, has efficacy for *Toxoplasma gondii* (*T. gondii*) vaccine to induce protective immunity in a mouse model.

Methods: Female BALB/c mice divided into five groups were immunized with excretory-secretory antigens (ESA), alum-ESA, PRP-ESA, and alum-PRP-ESA, as well as with phosphate-buffered saline (PBS) as the negative control group. The immune responses were evaluated by lymphocyte proliferation assay, measuring delayed-type hypersensitivity (DTH) response, and by cytokine assay for measuring IFN- γ and IL-5 levels. The survival rate of mice in all the groups was assessed during a three-week monitoring period after an intraperitoneal challenge with *T. gondii* RH strain tachyzoites.

Results: The results showed that immunization with PRP could lead to the secretion of a higher level of IFN- γ that was significant when compared with the data from other groups. However, alum-precipitated ESA vaccine had ability to produce an elevated level of IL-5 in comparison with other groups of mice ($p \leq 0.05$). We found more protection from alum-PRP co-administration with the vaccine since the mice receiving ESA vaccine plus the alum-PRP mixture showed longer survival.

Conclusions: This study reveals that the combination of alum-PRP and ESA vaccine of *T. gondii* elicits both humoral and cellular immune responses are comparable to either alum or PRP alone.

Keywords: *Toxoplasma gondii*, Propranolol, Alum, Vaccine, excreted/secreted antigens, IFN- γ and IL-5.

Preparation of Recombinant Antigens rK26, rK39 Derived Iranian Strain of *L. infantum* and Their Evaluation (alone and mixed) for Rapid Detection of Visceral Leishmaniasis in Animal Reservoir Hosts

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Introduction and goal: Mediterranean type of visceral leishmaniasis (VL) is a zoonotic parasitic infection transmitted through the bite of female *Phlebotomus* from animal reservoirs to human. Some provinces of Iran are endemic for VL including Ardabil, Azarbaijan-e-Sharghi, Korasan-e-Shomali, Fars, Kerman, Bousher, khuzestan while other parts are considered as sporadic areas. The main goal of this study was preparation of recombinant antigens rK26, rK39 derived Iranian strain of *L. infantum* and their evaluation (alone and mixed) for rapid detection of visceral leishmaniasis in animal reservoir hosts.

Material and method: In this study, rK26 and rK39 antigens of *L. infantum*, produced and purified in *E. coli*. The rK26 and rK39 genes of Iranian strain of *L. infantum* MCAN/IR/14/M14 (accession number KT201383) amplified and then ligated into pBluescript II SK (+) and pCR-TOPO vectors, respectively. The purified products after digestion were subcloned into the pET-32a (+) with histidine-binding protein and transformed into *E. coli*. Finally, the purified proteins after induction (Alone and Mix) were evaluated by ELISA method on 87 confirmed VL-infected sera ($\geq 1:80$) along with 90 healthy control and also cross-reactivity checked with other infectious diseases including toxoplasmosis and toxocariasis. The results of ELISA using recombinants and crude antigen-ELISA were compared to DAT as the gold standard and then analyzed by MedCalc and SPSS softwares.

Results: The sensitivity of these recombinants in dogs was observed 90.8% for rK26 and 96.6% for rK39 with specificity of 78% and 94.4%, respectively ($\geq 1:80$). The positivism rate of rK26 mixed rK39-ELISA test was 98.9% in dogs. The higher specificity was determined for diagnosis of CVL (96.7%) with the mixed recombinant antigens. The CA-ELISA test showed 93% sensitivity and 83.3% specificity. The kappa index was calculated for all the antigens to find the level of agreement with DAT. This value for rK26-ELISA, rK39-ELISA, rK39 mixed rK26-ELISA and CA-ELISA was 0.695, 0.910, 0.955 and 0.763

Conclusion: On the whole, rK39 and rK26 antigens have appropriate immune-dominant epitopes for diagnosis of VL. Not only the highest sensitivity and specificity were related to using rK39 and rK26 together, but also this test had the best agreement with DAT. The results indicate that combination of rK26 and rK39 could be effective for diagnosis of VL in infected cases.

Keywords: Recombinant antigen, rK39, rK26, Serology, Direct agglutination test, visceral leishmaniasis, Dog.

Molecular evidence of hypobiosis of parasitic nematodes larvae in sheep abomasum in southwest of Iran

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Introduction: Arrested development larvae of parasitic nematodes of sheep abomasum including *Dictyocaulus filarial*, *Marshallagia marshalli*, *Trichostrongylus colubriformis* and *Nematodirus filicollis* have been reported experimentally from south of Iran. The aim of the current research was molecular study of hypobiosis occurrence in nematodes of sheep abomasum.

Methods: A total numbers of 240 abomasa were randomly collected from Ilam slaughterhouse monthly in 2017-2018. The collected abomasa were washed and tissue specimens were taken from different 6 anatomic regions to do peptic digestion and DNA extraction. A 300 bp fragment of ITS2-rRNA gene was amplified using polymerase chain reaction (PCR) technique.

Results: Peptic digestion revealed there were no arrested development larvae. However, molecular evidence was proven the presence of hypobiotic larvae of *M. marshalli* and *T. circumcincta* during autumn and early winter.

Discussion & Conclusion: The highest strongyloid nematodes hypobiosis, i.e. *T. circumcincta* (87.7%) reported from Czech Republic during winter (Langrova *et al*, 2008). Meradi and coworkers (2016) also reported strongyloid nematodes hypobiosis from steppe region of North-East of Algeria during cold winter (20.5%) and hot-dry summer (48.3%). The findings indicated environmental condition for hypobiosis of parasitic nematodes of sheep abomasum was hospitable in the region and molecular tools were reliable than peptic digestion technique.

Keywords: Hypobiosis, Nematode, Sheep

Molecular and Parasitological Study of *Toxocara* Spp. in *Lumbricus Terrestris* Earthworms

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Background and Objectives: Soil-associated invertebrates which are coprophagic are capable of acting as paratenic hosts for *Toxocara* species larvae eggs. The aim of the present study was to assess the role of *Lumbricus terrestris* earthworms as potential intermediate host for *Toxocara* species.

Materials and Methods: A total of 240 earthworms were collected from the public places in four areas of Karaj, Alborz Province, Iran. The subjects of the study were digested to detect *Toxocara* species using microscopy and molecular analyses.

Results: *Toxocara* larvae and larval eggs were recovered from 1.7% (4/240) of the sampled earthworms. The recovery rate in fourth region in the studied areas was higher than that of the other regions. In the mentioned area, 8.3% (4/48) of earthworms were revealed to be positive for *Toxocara* species by microscopic observation and only one was confirmed to be positive for *Toxocara canis* by polymerase chain reaction (PCR).

Conclusion: This is the first molecular report of *Toxocara canis* infective larvae in *Lumbricus terrestris* earthworms that could be demonstrated in common environment.

Keywords: Earthworms, *Lumbricus terrestris*, Molecular study, *Toxocara* spp.

Prevalence and Anthelmintic Resistance in Gastrointestinal Nematodes of Sheep in Iran: A Systematic Review and How Overcome it

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Gastrointestinal nematodes (GIN) are an important cause of production losses in young grazing sheep, particularly in intensive production systems. The prevention of GIN infections and parasitic gastroenteritis relies on broad spectrum anthelmintic drugs. At present, the major classes of anthelmintics available for sheep belong to the families of the benzimidazoles (albendazole, febendazole, and oxfendazole), imidazothiazoles (levamisole) and macrocyclic lactones (ivermectins). Anthelmintic resistance has been recognized in small ruminants worldwide, and this phenomenon has increased to become a major worldwide problem to livestock industry.

A systematic review was conducted with the aim to measure the prevalence of sheep gastrointestinal nematodes (GIN) in Iran and potential management factors associated with development of anthelmintic resistance.

Modern strategy for nematode control should not rely on sole use of anthelmintics. Targeted selective treatments attract the interest of parasitologists towards maintaining parasites in refugia, seems to be a key point in controlling and delaying the development of resistance. Therefore, it is needed to use novel non-chemical approaches that decrease the need for treatment.

Seroprevalence of Toxocariasis among Municipal Street Sweepers in Shiraz District in Fars Province, Southern Iran

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Background and aim: Sweeping the street is a hazardous job that exposes the workers to soil-transmitted diseases, in particular, toxocariasis. Considering the importance of awareness of the seroprevalence of toxocariasis in street sweepers, the current study aimed to assess the seroprevalence of toxocariasis among street sweepers in Shiraz, southern Iran.

Materials and methods: Subjects of the study were 384 street sweeper selected by simple random sampling in 2018. Blood samples were collected from each individual and evaluated for antibodies to *Toxocara* by an enzyme-linked immunosorbent assay (ELISA) system.

Results: Anti-*Toxocara* antibodies were detected in sera of 101 (26.3%) out of 384 subjects. Most of the seropositive cases were in the age group of 31 to 50 years, yet no significant difference was found between age and *Toxocara* seropositivity ($P > 0.05$). Out of 101 seropositive cases, 21 (20.8%) had a work experience of less than 5 years. However, no significant association was observed between work experience and *Toxocara* seropositivity.

Conclusion: Findings of our study represents that street sweepers are at high risk of infection with toxocariasis. Therefore, appropriate guidelines for preventive measurements should be taken to reduce the risk of infection in this vulnerable group.

Keywords: *Toxocara*, Street sweepers, Fars Province, Iran

Toll-like Receptors 2, 4, and 9 and Cytokines Expression In peripheral Blood derived monocytes from Glucantime non-Healed and Healed patients with Anthroponotic Cutaneous Leishmaniasis Due to *Leishmania Tropica*

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Background: Anthroponotic cutaneous leishmaniasis (ACL) due to *Leishmania tropica* is a neglected disease which may develop to a non-healed form due to unknown reasons. Detection of the mechanism of host/parasite interactions in non-healed forms is critical for designing the new therapeutic approaches. In the present study, the expression of Toll-like receptors (TLRs), TNF- α and IFN- γ in monocytes from Glucantime non-healed in comparison to healed patients infected with *L. tropica* was investigated.

Methods: In this study, 10 patients with non-healed CL, 10 with healed CL and 5 healthy controls were recruited. Gene expression of TLR2, TLR4, TLR9, TNF- α and IFN- γ was analyzed in unexposed and *L. tropica*-exposed monocytes by means of quantitative real-time PCR.

Results: *L. tropica*-exposed monocytes represented higher expression of all three TLRs, TNF- α and IFN- γ compared to unexposed ones in both groups of patients. Results revealed a significant down-regulation of TLR2 1.9-fold ($p = 0.04$), TNF- α 1.9-fold ($p = 0.04$) and IFN- γ 2.5-fold ($p = 0.01$) and up-regulation of TLR9 expression 2.2-fold ($p = 0.04$) in non-healed isolates in comparison to healed ones. A positive correlation was identified between TLR2 and TLR4 expression ($p = 0.04$). Besides, a negative correlation was observed between TLR2 and TLR9 expression; but it was not statistically significant.

Conclusions: The decreased TLR2, TLR4, TNF- α and IFN- γ and the increased level of TLR9 expression in *L. tropica*-exposed monocytes from non-healed isolates might possibly be involved in the severity of the disease and leading to Glucantime unresponsiveness.

Keywords: Leishmaniasis, *Leishmania tropica*, unresponsiveness, Toll like receptors, inflammatory mediators

Evaluation of Eytotoxic Effects of Zinc Oxide Nanoparticles on Tachyzoites of *Toxoplasma Gondii* RH Strain at Experimental Conditions

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Introduction: *Toxoplasma gondii* is an obligate parasite with worldwide distribution, infecting wide range of humans and warm-blooded animals. This study was carried out to evaluate the effect of zinc oxide nanoparticles on tachyzoites of *T.gondii* RH strain.

Methods: The efficacy of various concentration of zinc oxide nanoparticles was assessed by performing in vitro experiments using trypan blue dye and scanning electron microscopy. Bioassay test was done in BALB/C mice.

Results: The results of in vitro and in vivo revealed that zinc oxide nanoparticles was effective against *T.gondii* tachyzoites. It has been confirmed from SEM images of the tachyzoites.

Conclusion: Zinc oxide nanoparticles can be used single or combined as an alternative agent in the treatment of toxoplasmosis.

Keywords: *Toxoplasma gondii* RH strain, zinc oxide nanoparticles

Using Molecular and Morphological Study in Northwestern Iran

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Background and objectives: Mosquito are potential vectors of some important diseases for humans and animals. We assessed the susceptibility of Culicidae mosquitoes found in Ahar County, East Azerbaijan Province and northwestern Iran, to natural infections of *Dirofilaria immitis* (canine or dog heartworm).

Methodology: Mosquitoes were collected in and around three fix selective villages in Ahar County: Afil (mountainous area), Noghdose (plain area) and Behel (foothill area) and six villages were selected as variable. Four standard sampling methods, hand collection, bed net trap, light trap and larval collection, were carried out from April 2016 to end of March 2017. Females *Cx. theileri* were dissected, and the parasites were detected. DNA of female species and its parasites isolated were extracted and amplified using ITS2-rDNA gene for PCR analyses and sequencing.

Results: Seven species of Culicidae mosquitoes were collected, including: *Anopheles claviger*, *An. persiensis*, *An. sacharovi*, *Culex hortensis*, *Cx. pipiens*, *Cx. theileri* and *Culiseta longiareolata*. Among the 274 Culicidae mosquitoes dissected, a total of 11 *Dirofilaria*-positive samples were detected. Natural *D. immitis* infection in *Cx. theileri* was reported in Iran.

Discussion and conclusion: This is the first report of a natural *D. immitis* infection of *Cx. theileri* in East Azerbaijan Province, northwestern Iran. The existence of the life cycle of the parasite in the vicinity of human habitats results in a significant risk of human infection. Further studies on *D. immitis* infection in humans and dogs are recommended in East Azerbaijan Province.

Key words: *Culex theileri*, *Dirofilaria immitis*, Internal transcribed spacer 2 (ITS2), Iran

Detection of glutathione S- transferases epsilon 2 gene in *Anopheles maculipennis*

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Objectives: The effectiveness of insecticide-based *Anopheles* vector control methods (insecticide-treated nets, indoor residual spraying, and larvicides) is treated by insecticide resistance. Glutathione S-transferases (GSTs) are considered as one of the effective factors in the occurrence of insecticide resistance. In the current study, sequences of GSTe2 were analyzed in *An. maculipennis* in northwestern Iran.

Materials and Methods: Adult samples of *An. maculipennis* were collected by hand and total catch in Animal and human Shelters. Morphological identification was confirmed by rDNA- ITS2 marker. GSTe2 fragment was amplified using specific primers. Sequence analysis was carried out using bioinformatics software.

Results: rDNA-ITS2 sequence analysis showed %100 similarity with *An. maculipennis* s.l. GSTe2 nucleotide sequence similarity within species was % 99.28- 100, whereas it was 95-96% with *Anopheles sacharovi* GSTe2 sequence available in GenBank. Amino acid sequence comparisons showed a novel amino acid substitution in N148D position except well-known I114T, F120L, and L119F mutations with % 15.8 frequency.

Discussion: The current study reports new GSTe2 amino acid substitution in *An. Maculipennis* for the first time. This finding could be provided an essential tool for monitoring insecticide resistance in malaria vectors management. However, the integration of these data into the malaria control program still remains a challenge.

Keywords: *Anopheles maculipennis*, Glutathione S-Transferases epsilon 2, polymorphism, novel mutation

The Potential Role of Toll-Like Receptor 4 Asp299Gly Polymorphism and Its Association with Recurrent Cystic Echinococcosis in Postoperative Patients

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Background: The study of pathogenesis mechanisms of larval stages in the Taeniidae has recently focused on host genetic factors, particularly toll-like receptor (TLR) variations. However, the potential role of TLR4 polymorphism in hydatidosis has not yet been sufficiently elucidated in postoperative patients.

Methods and Materials: In this case-control investigation, 80 patients from Iran, including 40 with acute hydatidosis (AH) and 40 with recurrent hydatidosis (RH), and 80 ethnically matched controls were evaluated from February 2015 to February 2017. Hydatidosis patients were confirmed using radiological, immunological, and histopathological examinations. Genotyping of Asp299Gly and Thr399Ile of TLR4 single-nucleotide polymorphisms was determined by restriction fragment length polymorphism, sequencing, and phylogenetic strategies.

Results: The heterozygous mutant-type TLR4 Asp299Gly genotype indicated a tendency to be associated with the occurrence of RH ($P=0.060$) and conferred a 3-fold risk for susceptibility. There was no difference in genotype frequency of Asp299 Gly between patients with AH and healthy controls ($P=0.42$; OR, 1.82; 95% CI, 0.11–30.1%). Interestingly, a frequency of the G allele (12%: Gly) was observed to be a risk factor for susceptibility to RH patients ($P=0.050$; OR, 7.08; 95% CI, 0.97–51.5%). A relative genetic variability of TLR4 Asp299Gly was found in RH patients (haplotype diversity: 0.700) compared to AH patients and healthy controls (Hd: 0.000). The Asp299Gly genotype was dominantly identified in patients with hepatic hydatid cysts. The TLR4 Thr399Ile codon was not detected except in a patient with a pulmonary hydatid cyst.

Conclusion: The current findings enhance our knowledge regarding the TLR4 Asp299Gly polymorphism potentially leading to the development of RH, by skewing the immune system towards a Th2 response. Identification of the Asp299Gly codon may be a diagnostic hallmark in RH patients who have undergone unsuccessful postoperative intervention. However, further studies with a higher case number are needed on ethnic population from various geographic regions, in order to confirm this hypothesis.

Key words: Recurrent hydatidosis, Innate immunity, Toll-like receptor 4, Unsuccessful postoperative intervention

Molecular Phylogenetic and Genetic Variability of *Fasciola hepatica* with an Overview to Understand Haplotypes Distribution in Iran

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Background: Fasciolosis is a zoonotic disease caused by *Fasciola hepatica* and *Fasciola gigantica*. Over the last decade, diagnostic tools to detect and differentiate *Fasciola* species have improved, but our understanding of the distribution of haplotypes and gene flow of this parasite is less clear.

The purpose of this work was to investigate this gap in the *F. hepatica* epidemiology in different provinces of Iran between 2015 and 2018.

Methods: Isolated *Fasciola* were collected from abattoirs in 9 provinces. The partial sequence of mitochondrial NADH dehydrogenase subunit 1 (*ND1*) gene was used for the identification and molecular analysis of *Fasciola hepatica* isolates. The amplified PCR products were purified and subjected to direct sequencing for subsequent construction of phylogenetic tree and network analysis.

Results: In the 130 individuals analyzed, 37 ND1 haplotypes were detected. The haplotype network showed a complex and moderately diversified topology.

This is the first study of *F. hepatica* population study and genetic structure, that maintain the diverse haplotypes of this parasite based on mitochondrial ND1, from different geographical regions of Iran. It is well documented that the genetic diversity of parasites can regularly happen following the high gene migration which expands the effective population size in a variety of geographical regions where the heterogeneity traits are potentially dominant.

Keywords: *Fasciola hepatica*, ND1 gene, Genetic diversity, Haplotypes, Iran

Evaluation of TH17 Immune Responses after Immunization with DNA Vaccine Encoding ROP13 Gene against *Toxoplasma gondii* in BALB/c Mice

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Background and aim: *Toxoplasma gondii*, an intracellular parasitic protozoan, is capable of infecting man and all warm blooded animals. Cell-mediated immunity is vital in mounting protective responses against *T. gondii* infection. DNA vaccines have shown with potential findings in this regard. Recent studies have shown that T-helper (Th) 17 responses may play a key role in parasite control.

Materials and methods: In this current study, we constructed a DNA vaccine encoding *T. gondii* ROP13 in a pcDNA vector. Groups of BALB/c mice were immunized intramuscularly with pcROP13 and challenged with the RH strain of *T. gondii*.

Results: The results showed that immunization with pcROP13 could elicit an antibody response against *T. gondii*. The expression of the canonical Th17 cytokines, IL-17 and IL-22, was significantly increased after immunization with pcROP13 compared with control groups ($p < 0.05$).

Discussion: The present study showed that ROP13 DNA vaccine induced a *T. gondii* specific immune response and it could be considered as a potential vaccine approach for the control of toxoplasmosis.

Keywords: *Toxoplasma gondii*, DNA vaccine, interleukin (IL)-17, ROP13, T-helper (Th) 17

Evaluation of Genotypic Frequency and Diversity of *Hymenolepis nana* Using the PCR-Sequencing Method

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Background: *Hymenolepis nana* (*H. nana*) or dwarf is a cosmopolitan cestode causing hymenolepiasis, which is among the commonest infectious parasitic diseases. Owing to the high prevalence of *H. nana*, it is one of the most common tapeworms, but there is not much knowledge about its biological and molecular characteristics.. Hence, owing to inadequate knowledge about the molecular characteristic of *H. nana* in Sistan region, Iran, the current study aimed at evaluating the mitochondrial genome of the parasite and genomic diversity.

Materials and Methods: *Hymenolepis nana* infection in children referring to medical laboratories in Zabol city was confirmed by the detection of parasite eggs in fecal samples, extraction of DNA from eggs by molecular techniques using the specific primers of small subunit ribosomal RNA (rrns) and cytochrom c oxidase (cox1) mitochondrial genes, and visualization of the gene bands. Then, the genes were sequenced and complete in rrns.

Conclusions: The current study results indicated that in spite of genome alignment similarity between *H. nana* in this region and other isolated from other areas of the world, but there were some differences in specific part of genome and complete genome of the previously identified species and those found in the current study too; however, such differences and similarities show specific characteristic that may need obvious procedure for control, drug resistance, or prescription and transmission.

Keywords: PCR; Sequencing; *Hymenolepis nana*; Gene

Molecular Characterization of *Acanthamoeba* Strains Isolated from the Oral Cavity of Hemodialysis Patients in Iran

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Introduction: Free-living amoebae (FLA) of the genus *Acanthamoeba* are opportunistic pathogenic agents able to cause life-threatening infections in immunosuppressed patients. Chronic kidney disease impairs adaptive and innate immunity. Thus, patients with chronic kidney disease are prone to opportunistic infections by potentially pathogenic FLA. In the present study, we have investigated *Acanthamoeba* genotypes isolated from the oral cavity of hemodialysis patients of reference hospitals in Iran.

Material and methods: Samples from the oral cavity of hemodialysis patients were collected in different hospitals of Tehran, Iran using sterile cotton swabs. Samples were cultured in 2% Non-Nutrient Agar (NNA) plates. Identification of isolates was carried out using both morphological and molecular tools. Classification of the strains at the genotype level was performed on the basis of differences in the diagnostic fraction 3 region of the 18S rRNA gene. The pathogenic potential of the isolated amoebae was also determined using thermotolerance and osmotolerance assays.

Result: Out of the 187 oral cavity samples, nine (4.8%) were positive for FLA. DNA sequencing revealed that the isolated strains belonged to the *Acanthamoeba* T1 (1 isolate) and T4 (6 isolate) genotypes. Interestingly, the T1 strain exhibits a high pathogenic potential in tolerance assays. The pathogenicity assay revealed that five strains were able to grow at high temperatures and high osmolarity conditions; thus, they were considered as potentially pathogenic strains. Moreover, two of the patients were positive for *Vermamoeba* genus.

Discussion: Monitoring hemodialysis and renal failure patients should be a priority for possible control of *Acanthamoeba* and other FLA-related diseases.

Keywords: *Acanthamoeba*, Hemodialysis patients, Iran

A Low-Cost High-Precision Molecular Detection Method of Toxoplasmosis in Developing Countries

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ABSTRACT: *Toxoplasma gondii* is an obligate intracellular protozoan with worldwide distribution. Diagnosis of toxoplasmosis is a very critical issue, especially in pregnant women and immunocompromised patients. The aim of this study was rapid detection of *T.gondii* DNA in peripheral blood samples (PBS) employing HRM technique and using RE gene. Totally two hundred and forty-two samples from pregnant women and human immunodeficiency virus (HIV) patients were collected. High resolution melting analysis (HRM) using partial sequences of repetitive element (RE) gene was done and compared with enzyme-linked immunosorbent assay (ELISA) test. The data were analyzed by MedCalc and SPSS Statistical Software and compared with ELISA as reference test. Out of 242 collected samples, 51 were samples with acute phase of toxoplasmosis that among them, twelve and twenty-two were reported as positive for pregnant women and HIV⁺ patients, respectively, also out of 70 patients in chronic phase of the disease, ten and three samples were reported as positive for pregnant women and HIV⁺ patients respectively. The notable point was that among 121 negative control, 3 (4.62%) samples associated with HIV⁺ patients, showed positive real-time PCR and HRM analysis results. For the first time, HRM technique via employing RE gene was used for detection of *T.gondii* infection in PBS, this method is suitable, helpful and in parallel of serological methods for early diagnosis of acute as well as active form of toxoplasmosis in pregnant women and HIV⁺ patients. It is hoped that in the not-too-distant future use of techniques based on melt curve and through employing next-generation dyes for diagnosis of *T. gondii* would be accessible for patients in developing countries.

Key word: Toxoplasmosis, Pregnant women, HIV⁺ patients, HRM, Real-time PCR, and Melt curve.

Microanatomical Identification of Helminths in Tissue Sections with Focus on Fasciolid Worms

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Introduction: Fascioliasis is among the neglected diseases throughout the world. In Iran however infection by *Fasciola* liver flukes are known endemic. Due to the eco-biological conditions in the country infection transmission have been reported for the both species so far. Identification of the adult worms meanwhile could not be relied merely of old fashion morphological techniques. Taking advantage of computer imaging analysis system (CIAS) has provided an important occasion for the parasitologists on the scene of taxonomy. In present study, tegumental spines of the both species were successfully implemented for species identification in tissue sections.

Methods: The adult worms were collected from two slaughterhouses, and were divided preliminary in two groups based on their apparent features. RFLP-PCR technique were also performed to confirm the diagnosis. To observe our hypothesis in sections, histopathological slides were obtained. To complete the morphological values in species differentiation Scanning Electron Microscopy (SEM) has been also carried out

Results: Molecular studies verified 10 samples as *F. hepatica* and 37 as *F. gigantica*. All samples, initially recognized as *F. hepatica* and *F. gigantica* were matched based on molecular criteria. In tissue section, morphological and morphometrical characters of tegumental embedded spines were seen different in two species, so that the spines of *F. hepatica* were prominently pointed in shape compared with those of molar spines in *F. gigantica* and the length of the spines were seen significantly different in two species ($P < 0.001$), with taller ones for *F. hepatica*.

Conclusion: The present study reveals the capability of a non-molecular tool that can be utilized in species identification between *F. hepatica* and *F. gigantica*.

Keywords: *Fasciola*, Tissue section, Diagnosis, Tegumental spine

Changes in Splenocytes Proliferation activity and Cytokine Production in Mice Immunized with Recombinant Plasmid, Encoding EG95 (G1) Antigen of *Echinococcus granulosus*

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Introduction: Hydatidosis as an important zoonotic disease, cause to economic losses and health problems. Interruption of the parasite life cycle within vaccination of the intermediate host has been recommended as a control strategy. EG95 is a protective antigen of *E. granulosus* and EG95 recombinant vaccine was developed in 1996 which induced high protection in carried out vaccine trials. This study was designed to evaluate the DNA vaccine formulation of EG95 antigen.

Materials and Methods: In current study, the recombinant plasmid encoding EG95 protein was successfully constructed. BALB/c mice were vaccinated three times at 2 weeks intervals with recombinant pcDNA3.1 encoding EG95, empty pcDNA3.1 and saline in experimental and control groups respectively. Lymphocyte proliferation, IFN- γ , IL-4 and IL-10 levels were measured in all groups 5 weeks after the last immunization and IgG1, IgG2a and total IgG levels were determined in collected sera to evaluate induced immune responses. Therefore spleens from immunized mice were removed and splenocytes were cultured with *E. granulosus* crude antigen to lymphocyte proliferation assay and to measure cytokine level.

Results: This study indicated that lymphocyte proliferation was significantly higher in mice immunized with pcDNA3.1-EG95 ($p < 0.05$). Culture supernatant cytokines from the mice vaccinated intramuscularly with pcDNA3.1-EG95 demonstrated preferential production of IFN- γ , which was significantly higher than that of control groups ($p < 0.05$). There was no statistically significant difference in level of IL-10 and IL-4 between vaccinated groups and control groups ($p > 0.05$). Higher levels of IgG1, IgG2a and total IgG antibodies were detected in immunized mice compared to control groups ($P < 0.05$).

Conclusion: The results indicated that the pcDNA3.1-EG95 recombinant plasmid could induce Th1 immune responses.

Keywords: DNA vaccine; *Echinococcus granulosus*; EG95

Genetic Diversity of *Plasmodium vivax* from Three Geographical Locations: Molecular Study Followed by Bioinformatics Analysis

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Background: Despite decreases in incidence, Malaria has been remaining a serious infectious disease and *Plasmodium vivax* (*P.vivax*) species is predominant causative of disease with about 90% of annual reported cases in Iran. This study conducted to determine genetic diversity of *P. vivax* merozoite surface protein-1 (*Pvmsp-1*) at three geographical locations in order to evaluate impacts of diversity on disease control.

Methods: In this study, 129 positive blood samples were collected from three neighboring countries: Iran, Pakistan and Afghanistan during 2015-2018. Genomic DNA was acquired and fragments of *Pvmsp-1* were amplified and sequenced. The sequences of *Pvmsp-1* obtained from the studied regions and were compared with homologous sequences from the GenBank database to explore the *P. vivax* genetic structure.

Results: Results of PCR products of *Pvmsp-1* gen with *Pvmsp-1* specific primer showed 1124bp long band. Genotyping of *pvmosp-1* gen revealed the presence of three type haplotypes with type1 predominant. The most of the polymorphisms were found within the 400-600 bp region. Some of the Iranian *P.vivax* isolations showed 98% similarity in their identity in comparison with other strains.

Conclusion: The study of genetic diversity distribution in Malaria, shows information that is pivotal to Malaria control and elimination programs. Nevertheless the high homology among this geographical regions, reveals difference haplotypes between Iranian and others samples, it can be concluded that transmission of malaria disease in Iran is local.

Keywords: Msp-1 gene, *P.vivax*, Homology, Bioinformatics.

Identification and distribution of mosquitoes, Urmia City, West Azerbaijan province, Northwestern Iran

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Objectives: Mosquitoes are the most medically important arthropods that cause global public health problems. They belong to the family Culicidae, with worldwide distribution. These insects can transmit several important diseases such as Malaria, Yellow fever, Filariasis, West Nile fever, Dengue and Dengue hemorrhagic fever. Current study was performed in order to identify the adults and larva of mosquitoes in Urmia city and determine their distribution across the study areas.

Materials and Methods: Mosquito specimens were collected using previously described standard methods. Larvae were collected from larval habitats with dipping technique and adults were collected with light traps. The species identification was based on morphological characters using standard keys.

Results: In current study, specimens from 3 region of urmia city that belonging to 3 genera (*Culex*, *Anopheles* and *Culiseta*) and 5 species (*An. maculipennis*, *Cx. pipiens*, *Cx. theileri*, *Cx.modestus*, and *Cs. longiareolata*) of the family culicidae were collected and identified. Finally the distribution of identified species were determined according to geographical properties of collection sites.

Conclusion: According to the history of mosquito-borne-diseases in Iran and especially studied region, the identification of mosquito in these areas is of great importance. The identified species can transmit various pathogens including *Plasmodium* parasites.

Keywords: Vector Borne Diseases, Malaria, Mosquitoes, Culicidae.

Geographical Distribution of Tick Vectors of Theilriosis and Babesiosis in Livestock in Large Khorasan, Iran

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Backgrounds: Babesiosis and theileriosis are important tick-borne diseases in Iran. Several molecular studies were carried out to identify tick vectors of Theilriosis and Babesiosis in cattle, sheep, goats and horses in different areas in large Khorasan from 2003- 2016.

Methods: A total 3924 Ixodid ticks were collected from different areas of large Khorasan. The collected ticks were separated into tick pools, according to their species and sex. Then, their salivary glands and ovaries were dissected out in 0.85% saline under stereomicroscope and analyzed by semi-nested PCR.

Results: Eleven Ixodid tick were identified as follow : *Rhipicephalus turanicus* 52.2 % , *Hyalomma excavatum* 19.9 % , *Hyalomma anatolicum* 12.52 % , *Hyalomma marginatum* 11.4% , *Dermacentor marginatum* 1.67% , , *Rhipicephalus bursa* 0.77 , *Hyalomma asiaticum* 0.69 , *Dermacentor raskemensis* 0.54 , *Dermacentor niveus* 0.36% , *Rhipicephalus sangienseus* 0.12 and *Haemaphysalis sulcata* 0.05%. The genomic DNA of *Theileria* spp was detected in salivary glands of *H.excavatum*, *H.anatolicum*, *H.marginatum*, *R. turanicus* and *R. bursa*, while *Babesia* spp was detected in *R.turanicus* and *H.marginatum*

Conclusion: The results suggest that *H.excavatum* could be as vector of *T.annulata* and *T.equi*, *H.anatolicum* as vector *T.annulata* and *T.lestouardi*, *H.marginatum* and *R. turanicus* as vector of *T.ovis* and *B.ovis* and *R.bursa* as vector of *T.equi* .

Keywords: Tick vector; *Theileria*; *Babesia*; Livestock; Khorasan

Bed Bugs from Different Geographical Regions of Iran

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Introduction & aims: Bedbugs are blood feeding ectoparasites of humans and domesticated animals. There are two common species of bedbugs, *Cimex hemipterus* and *Cimex lectularius* which have a wide distribution in tropical and subtropical countries respectively. There are scarcity of information about the bed bugs population throughout Iran and only very limited studies are available. Aims of this study were to assess the phylogenetic relationships and genetic diversity using partial sequences of cytochrome oxidase I gene (COI) among the populations of bed bugs in Iran.

Materials and Methods: Bedbugs were collected from various places such as hotels, residential houses and industrial buildings from four different geographical regions of Iran (Caspian Sea region, mountainous area, Persian Gulf area and desert area) from May 2016 to August 2017. All samples were identified using identification keys. For phylogenetic analysis, after DNA extraction, PCR was performed for amplification of COI gene using specific primers. Then DNA sequencing was performed on PCR products.

Results: Based on diagnostic keys, all specimens were identified as belonging to the species *C. hemipterus*. DNA sequencing analysis showed that the all *C. hemipterus* bugs were similar, despite the minor nucleotide variations. Subsequently, there were close similarity and sequence homology with *C. hemipterus* from other parts of the world.

Discussion: The current research was the first report of phylogenetic and genetic species diversity analysis conducted on *C.hemipterus* in Iran. These results provided basic information for further studies of molecular epidemiology, public health and control of *C.hemipterus* infestation in Iran.

Keywords: *Cimex hemipterus*, Genetic diversity, Phylogenetic analysis, Iran

Misdiagnosis of Cutaneous Leishmaniasis, a Matter of Concern

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Introduction: Different clinical presentations of cutaneous Leishmaniasis (CL) are classified as: regular or volcano form, lupoid, tuberculoid, erysipeloid, keloid, sporotrichotic, disseminated, diffused, verrucotic and herpes(zona) form etc., but still many CL lesions are not observed in typical form that may mislead the physician. Atypical lesions appear due to the following reasons: Impaired immunity, genotype of *Leishmania*, cytokine secretion, secondary bacterial infection, self-treatment by abuse drug and misdiagnosis which may lead to wrong diagnosis and improper treatment.

Method: In order to investigate misdiagnosed CL lesions, a study was performed in parasitology laboratory of Emam Reza Hospital, Mashhad, Iran. The records of all misdiagnosed cases of past 3 decades were obtained and analyzed.

Result: More than 250 patients with unusual CL presentation who were misdiagnosed enrolled in this study. Many of them had history of 3-4 years misdiagnosis. More than 65% of the patients were followed up until definite diagnosis obtained and proper treatment performed.

Conclusion: It is concluded that obtaining a definite case history is necessary before clinical and paraclinical examination. In endemic areas every persistent skin lesion should clinically diagnosed as CL.

Key words: Cutaneous Leishmaniasis, Misdiagnosis, Clinical Presentation, Mashhad

Molecular Evaluation of *pvdhfr* and *pvmdr-1* Mutants in *Plasmodium vivax* Isolates after Treatment with Sulfadoxine/Pyrimethamine and Chloroquine in Iran during 2015–2016

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Introduction: The rising use of sulfadoxine/pyrimethamine (SP) in the treatment of chloroquine (CQ)-resistant *Plasmodium falciparum* has resulted in increased exposure to *P. vivax* isolates in Iran, where both species are being circulated. In this investigation, the frequency of *pvdhfr* and *pvmdr-1* mutants was assessed in *P. vivax* strains during 2015–2016 after the introduction of SP/CQ in malarious areas of Iran.

Material and Method: The *P. vivax* isolates (n, 52) were obtained from autochthonous samples in Southeast Iran during 2015–2016. The genomic DNA was extracted and examined using nested polymerase chain reaction and sequencing.

Result: Mutations were detected in *pvdhfr* codons P33L (21.2%), T61 M (25%), S93H (3.9%), and S117 T (1.9%) and 5 isolates showed double mutations (33 L/61 M, 7.7%; 33 L/117 T, 1.9%). No mutation was identified in *pvdhfr* codons F57 and S58. The *pvmdr-1* 1076 L mutation was detected in 93.3% of *P. vivax* isolates.

Discussion: The findings indicated that the frequency of three codons of *pvdhfr* F57/S58/S117 has decreased from 2001 (1.05%/7.0%/16.9%) to 2016 (0%/0%/1.9%). Genomic analysis of *pvmdr-1* showed that the frequency of 1076 L has gradually increased from 2013 (93%) to 2016 (93.3%) ($P > .05$). The results demonstrated that *P. vivax* isolates are probably being exited under SP pressure, which reflects the appropriate level of training for field microscopists, as established by Iranian policymakers. Emergent *pvdhfr* codons 33L, 61M, and 93H should be noticed in plausible drug tolerance and treatment plans. The high prevalence of *pvmdr-1* 1076L mutation shows that efficacy of CQ combination with primaquine may be in danger of being compromised.

Keywords: *Plasmodium vivax*, Drug resistance markers, Polymorphism, Iran

The Role of MicroRNAs as New Diagnostic Biomarkers in Malaria Patients

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Introduction: Malaria is one of the most important infectious diseases in the world. Early diagnosis of Malaria is very important in choosing the appropriate therapeutic approach. Changes in the amount of microRNAs have recently been considered in infectious diseases. MicroRNAs are a group of small, non-coding RNAs that have 18 to 24 nucleotides. Their main role is to regulate gene expression. The main objective of this study was to identify four microRNAs in the serum of Malaria patients compared to healthy group.

Methods: This study was conducted in southeastern Iran in 2018. Patients who referred to health centers with clinical symptoms of Malaria and with *Plasmodium vivax* in their microscopic smears were participated in the study. Two milliliters of venous blood was taken from the patients. The nested PCR was used to confirm the infection with *Plasmodium vivax* using the 18S ribosomal RNA gene. Then, four microRNAs including Mir 223, Mir 191, Mir 155 and Mir 145 were measured in serum of patients as well as in healthy group using Real Time PCR.

Results: *Plasmodium vivax* was detected by PCR assay in all patients. In Real Time PCR, the values of three of four microRNAs mentioned above in malaria patients were significantly different from those of the control group ($P < 0.05$).

Conclusion: The amounts of microRNAs in the serum of Malaria patients were different from healthy group. Therefore, the evaluation of microRNAs as a new diagnostic biomarker can be used in suspected Malaria patients.

Key words: Malaria, Real time PCR, MicroRNA

Rapid Differential Diagnosis of Vaginal Infections Using Gold Nanoparticles Coated with Specific Antibodies

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Background: Vaginal infections caused by bacteria, *Candida* and *Trichomonas vaginalis* affect millions of women annually worldwide. Symptoms and signs have limited value in differential diagnosis of three causes of vaginitis. Current laboratory methods for differential diagnosis are either expensive or time consuming. So, in this work development of a method based on gold nanoparticles has been investigated for rapid diagnosis of vaginal infections.

Method: Specific antibodies against three main causes of vaginal infections were raised in rabbits. The antibodies were then purified and conjugated to gold nanoparticles and used in an agglutination test for detection of vaginal infections. Finally, sensitivity and specificity of this test for diagnosis of vaginal infections were estimated using culture method as gold standard.

Results: Purification of antibodies from sera was confirmed by electrophoresis. Construction of nanoparticles was proved by TEM and FTIR methods. Conjugation of antibodies to gold nanoparticles was confirmed using XPS method. Sensitivity and specificity of gold nanoparticles for diagnosis of *candida* species was 100% and for *Gardnerella* was 100% and 93% respectively.

Conclusion: Gold nanoparticles-based method is a simple, rapid, accurate and cost-effective test for differential laboratory diagnosis of vaginal infections.

Keywords: Gold nanoparticles, Antibody conjugated, vaginal infection, diagnosis

Parasites Antigens as Possible Targets for Cancer Therapy

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Abstract

There is an adverse relationship between incidence of parasitic diseases and cancers in human population indicating that parasites may have anti-cancer activities. There are many scientific evidences in animal model investigation or invitro works to support this idea. Larval stage of the tape worm *Echinococcus granulosus*, *Toxoplasma gondii*, *Trypanosoma cruzi*, *Plasmodiums* and *Trichinella spiralis* are among the parasites that have been subjects of anti-cancer researches in the last decades. Anti tumor activities of parasites may be due to direct effect of the parasite or parasite molecules or due to the immune response raised against common antigens between cancer and parasites. In this work, evidences about anti-cancer effects of parasites in human population, laboratory works and animal model researches have been reviewed. Finally, possible options for using parasites or their molecules for treatment of cancer in human in future have been discussed.

Keywords: parasites, anti-cancer, common antigens, cancer therapy

The serological study of *Toxocara* infection among patients referred to Educational and Therapeutic Centers affiliated to Alborz University of Medical Sciences, Karaj, Iran during 2018

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Background and Objectives: Toxocariasis is an infection predominantly caused by migration of the roundworm *Toxocara canis* larvae to organs and tissues. The major clinical consequences of prolonged migration of larvae in humans are visceral larva migrans (VLM) and ocular Toxocariasis (OT). In this study, we investigated the seroprevalence of and risk factors for *T. canis* infection among patients referred to Educational and Therapeutic Centers affiliated to Alborz University of Medical Sciences through screening for sera anti-*Toxocara* IgG antibodies by questionnaire interview and ELISA.

Materials and Methods: Through a cross sectional study design, the presence of anti-*Toxocara* IgG antibodies was determined in 234 patients referred to educational and therapeutic centers affiliated to Alborz University of Medical Sciences, Karaj Iran using an enzyme-linked immunoassay between March 2017 to 2018. Associations of *Toxocara* exposure with socio-demographic, work, clinical, and behavioral data of the patients were also evaluated.

Results: Of the 234 subject patients who participated in the study, 105 (44.87%) were male and 129 (55.13%) were female. The seroprevalence of anti-*Toxocara* IgG antibodies was positive in 14.1% (33/234). There was significantly higher proportion of exposure to dogs ($P=0.014$) and history of soil contact ($P=0.032$) among the seropositive subjects. There was no significant difference between

Toxocara infection in people studied and socio-demographic such as of age group, gender, salary, and education.

Conclusion: This is the first report of seroprevalence and contributing factors for *Toxocara canis* infection in population in Karaj, Iran. These data confirm the importance of Toxocariasis in these regions, needing further evaluation of the present risk factors.

Keywords: Seroepidemiology, *Toxocara canis*, Toxocariasis, ELISA, Alborz

Application of proteomics in Helminths

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Abstract: Helminths have a significant impact on the health and economy of communities, particularly in tropical and subtropical regions. Helminths have adapted to live in different niches, such as the liver, intestine, lungs, and blood vessels, and many of them undertake a multiorgan migratory path in the human host to reach their final destination. For this purpose, helminths secrete a range of molecules that facilitate their penetration, migration, and establishment in the host. Furthermore, helminths have developed a number of mechanisms allowing them to evade the host immune responses, shaping and skewing it towards a regulatory phenotype that ensures long-term parasite survival while causing as little damage as possible to the host. Parasitic helminth proteins therefore are at the forefront of research efforts for the development of control tools (drug targets and vaccines), and more recently as a novel source of drugs to treat inflammatory and metabolic disorders that are now rife in populations that are (almost paradoxically) free of helminth infections.

A proteogenomics approach, combining proteomics data with genome sequencing, can provide invaluable data for the annotation of genes and proteins of helminths. This is an exciting new approach in the field of helminthology that can help to identify novel peptides and proteins involved in host–parasite interactions. Protein–protein interaction analyses can provide valuable information for understanding the molecular host–parasite interface. Although the immunomodulatory properties of helminths have been appreciated for some time, characterization of the individual bioactive proteins exerting these effects is still in its infancy. Taking advantage of genomic and proteomic big data will capitalize on this unique opportunity to characterize the helminth-derived pharmacopoeia for allergic and autoimmune diseases.

Key word: Dendritic cells, allergic asthma, *Marshallagia marshalli*, IL-10, TGF- β

Seroprevalence of Toxocariosis in association with different risk factors among children of 4-12 years-old referred to some medical centers in Aras Free Zone, Northwest Iran

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Background: Toxocariosis is a parasitic disease caused by the larvae of dog and cat *Toxocara* species. It has worldwide distribution with higher prevalence in children. In this study, sero-prevalence of *Toxocara* infection and its association with some risk factors were evaluated among children of Aras Free Zone (Jolfa) in North West of Iran.

Method: Sera were collected from 514 children aged 4–12 years old attending to some medical centers in the study area between May 2018 to February 2019. Anti-*Toxocara* IgG antibody assay was performed using commercial ELISA kit (Nova Tec, Germany). Sero-positivity were determined, and its association with different demographic criteria and risk factors were statistically analyzed.

Results: The overall sero-prevalence was found 2.3% (12/514). Risk factors of children's age ($p = 0.02$) and contact with either pet animals (dog and cat) or soil ($p = 0.004$) were significantly associated with sero-positivity. However, there was not any relationship between *Toxocara* infection and gender of children, place of residency (urban or rural) and their mothers' education level.

Conclusion: Both girls and boys are at the risk of *Toxocara* infection in the study area. Younger age of childhood and contact with sources of infection were important associated factors. Attention of health authorities for screening the infection and preventive measures are required.

Keywords: Seroprevalence, Toxocariosis, risk factors, children, Aras Free Zone, Iran

Application of High Resolution Melting analysis (HRM) based on *Cox 1* gene on some human isolates of *Strongyloides stercoralis* from Iran

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Background: *Strongyloides stercoralis* is a parasite with special characteristics presenting it as a unique nematode. Speculation into its genetic basis needs diverse molecular assessments. Iran is an endemic area for *S. stercoralis*. In this study, some human isolates of *S. stercoralis* collected from this country were applied for qPCR-High Resolution Melting analysis (HRM).

Methods: 12 isolates of *S. stercoralis* were collected from four endemic provinces of Iran including Guilan, Mazandaran, Khuzestan and Hormozgan, three isolates for each one. Genomic DNA was extracted from single filariform larva for every isolate. Using specific primers targeting partial regions in *Cox1* gene, qPCR-HRM was performed and melting-curve profiles were analyzed alongside with DNA sequencing results.

Results: Melting temperature (T_m) values of the isolates were 77.9 °C-78.3 °C. All isolates from Guilan, Mazandara and Khuzestan Provinces shared T_m 78.2 °C to 78.3 °C, while the isolates from Hormozgan Province, showed T_m of 77.9 °C, 78.0 °C and 78.1 °C. The two former correspondent isolates of Hormozgan Province were placed in common phylogenetic clade with Guilan and Mazandara isolates while the later one with those of Khuzestan's was positioned in separate clade.

Conclusion: Isolates of *S. stercoralis* from Hormozgan Province, compared to other isolates, had lower mean of T_m . Moreover, these isolates distributed in two different phylogenetic clades, while each set of corresponded three isolates of other provinces positioned in unit clade.

Keywords: *Strongyloides stercoralis*, HRM, *Cox1*, Iran

Anti-*Toxoplasma* effect of TiO₂ nanoparticles and Persian Gulf brown algae on Tachyzoites of *Toxoplasma gondii* (RH Strain) Proliferation

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Background: One of the main concerns regarding Toxoplasmosis is the therapeutic constraints. Current drugs used against *Toxoplasma* cannot eliminate parasites and infections. The aim of this study was to evaluate the effect of titanium dioxide (TiO₂) nanoparticles (NPs) and methanolic extract of Persian Gulf brown algae (*Sargassum oligocystum*) on the growth and cell death of *T. gondii* tachyzoites in vitro and in vivo.

Methods: BALB/c mice were infected with *T. gondii* tachyzoites. Four days after treatment, the cytokines were tested for IFN- γ , and the levels of splenic lymphocyte proliferation were investigated, and the groups were challenged with *T. gondii*.

Results: The results showed that the combination of *S. oligocystum* extract and TiO₂ NPs had more cytotoxic potential compared to their use separately. The challenge test results revealed that mice treated with the combination of algae extract and TiO₂ NPs had the highest lifetime expectancy than those receiving alone.

Conclusion: The simultaneous use of immunomodulator compounds for the stimulation of immune system as well as herbal and TiO₂ NPs with antiparasitic capabilities and potency can be promising to introduce drugs to treat toxoplasmosis.

Keywords: *Toxoplasma gondii*, TiO₂ NPs, Persian Gulf brown algae

Genotype characterization of *Enterobius vermicularis* isolated from infected humans based on the mitochondrial cytochrome C oxidase 1 gene using the PCR-sequencing method in two selected parts of Iran

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Introduction: *Enterobius vermicularis* (pinworm) is the most common intestinal nematode in children that causes enterobiasis. Molecular studies have revealed the genotypes A, B and C in *E. vermicularis* from humans and chimpanzees. The objective of the present study was to characterize the genotype of *E. vermicularis* from adhesive tape samples in Iran using the PCR-sequencing method.

Material & Methods: Twenty-seven adhesive tape samples contained *E. vermicularis* eggs were used for genotype characterization of *E. vermicularis*. The adhesive tapes were cut in parts where the egg/s of *E. vermicularis* was observed and used for DNA extraction. Genomic DNA was extracted and mitochondrial cytochrome C oxidase subunit 1 (cox1) gene was amplified by the polymerase chain reaction. The amplicons were sequenced and compared with the GenBank reference sequences using BLAST system. Phylogenetic analysis was performed using the maximum likelihood method in the MEGA 5.0 software.

Results: An approximately 390 bp band was amplified from 27 isolates. PCR and sequence analysis was performed on 17 PCR products to characterize the genotype of *E. vermicularis*. BLAST analysis indicated that the sequenced isolates belonged to *E. vermicularis* genotype B while intra-species variation was observed in 0-1.2% samples.

Conclusions: The genotype B of *E. vermicularis* in humans was identified in our study and considered as the only predominant genotype of this nematode in these regions. For characterization of the genotype of *E. vermicularis* including its haplotypes, comprehensive molecular studies with a large number of *E. vermicularis* isolates are necessary.

Keywords: *Enterobius vermicularis*, Genotype, Cox1, Iran

Subclinical *Leishmania* Infection in Healthy Children and HIV-Infected Patients in the Endemic Areas of Visceral Leishmaniasis: A Big Challenge for VL Control

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Background and objectives: HIV/VL co-infection is a major health concern in VL-endemic areas of Iran. Moreover, the rates of subclinical *Leishmania* infection in healthy children living in VL-endemic areas are considerable. These asymptomatic healthy subjects may act as reservoir of VL. Through a line of studies we evaluated the rate of subclinical Leishmanial infection in children as well as in HIV-infected individuals in VL-endemic areas in Fars province, southern Iran, using serological and molecular methods.

Methods: In study on HIV infected patients, blood samples were obtained from 251 HIV-patients who were clinically asymptomatic for Leishmaniasis and evaluated for anti-*Leishmania* antibodies, using ELISA, or *Leishmania* DNA, using molecular method. In another study, 617 asymptomatic healthy children living in a VL-endemic area in Kazeroun, south of Iran, were assessed for antibodies against *Leishmania infantum* by an indirect ELISA and their buffy coats were tested for the presence of *L. infantum* DNA by molecular method.

Results: Using the serological and molecular methods, anti-*Leishmania* antibodies were detected in 13 (5.2%) and Leishmanial DNA in 8 (3.2%) of 251 HIV patients. The sequence analysis of DNA-positive cases revealed the species of the parasite as *L. infantum*. Of the 617 recruited children, anti-*Leishmania* antibodies were detected in 17 (2.8%) of the children and *L. infantum* DNA was detected in the buffy coat of 8 (1.3%) of the children.

Conclusion: As the PCR-positive healthy individuals may act as a reservoir for VL, the high prevalence of *Leishmania* infection in asymptomatic healthy children as well as in HIV-infected individuals in VL-endemic areas in Iran is a serious challenge in surveillance, prevention, and control of VL.

Keywords: Visceral Leishmaniasis, Subclinical, HIV/VL co-infection, Iran.

The effect of Somatic extract of *Toxocara canis* on airway inflammation in mice model

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Background: Asthma is a chronic heterogeneous disorder of airways and nearly 400 million people are affected worldwide. The intrinsic immune responses due to helminthic parasites and airways inflammation have destructive effects on epithelial cells, which leads to the release of a bunch of Alarmin molecules such as IL-25, IL-33 and TSLP. Former studies confirmed that Helminths can decrease alarmins. In the present study, we evaluated the expressions of Alarmin cytokine in the lung tissue of murine model of airway inflammation encountered with *T. canis* somatic extract.

Methods: A total of 30 female pathogen-free BALB/c mice aged 6–7 weeks contain one control group and two experimental groups (ten mice in each group) were purchased from the Razi Vaccine and Serum Research Institute. The control group sensitized with PBS+ Alum. The second group sensitized with OVA and the third group administered with 20 µg somatic extract of *T. canis* along with OVA + Alum and challenged with OVA. 24 hours after the last challenge. Lungs were removed and divided in two sections a piece of the right lung tissue for qPCR and remained lung tissue for the histopathological survey.

Result: The expression of cytokines of lung tissue in OVA + Alum + *T. canis* sensitized group was significantly lower than other experimental groups, ($p < 0.05$).

Conclusion: The antigens of this parasite contain many epitopes, which some of them can stimulate the immune system. The epitope mapping studies are very helpful because they can highlight the main candidate. So, first of all, these epitopes can be synthesized as a therapeutic product and also, they can be used in clinical trials. However, parasitic antigens have potential in the inhibition and treatment of airway inflammation, but there is a long way to go to elucidate the mechanism and discover more effective.

Keyword: Somatic extract, *Toxocara canis*, airway inflammation, mice model

In vitro clonal growth of *Giardia intestinalis*

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Introduction: Establishment of the axenic culture of *Giardia intestinalis* is the most important of the supply of the parasites. However, the fastidiousness of *giardia*, contamination and replacing fresh medium and antibiotics are some challenging for axenic culture of the parasite. In this study a method for clonal growth of *G. intestinalis* trophozoites in the semisolid agarose medium was developed to multiply of active and viable trophozoites.

Material and method: Concentrated stock solution (2.5% w/v) of melted agarose (Sigma) was prepared. Different volumes of this solution (including: 0.18, 0.22, 0.25 and 0.29%) were added to 10 ml of filter-sterilized Keister's modified TYI-S-33 medium (complete, without vitamins, and without serum). Then 1 ml of the axenic cultured medium containing the parasite was added to the prepared culture media. The contents mixed by inverting the tubes and incubated at 35.5 °C.

Result: Colonies become visible after 5 to 7 days' incubation. Clonal growth was found in TYI-S-33 medium with 0.25% and 0.22% of agarose volumes. The "attached" (to the walls of the culture tubes) were more than with "free" (swimming in the medium) trophozoites. In the tubes of TYI-S-33 medium without vitamins, the parasites were fewer and in the TYI-S-33 tubes without serum, the parasite was absent.

Conclusion: Clonal growth was depending upon the agarose concentration and TYI-S-33 media situation. Higher colony-forming of *G. intestinalis* was obtained with "attached" more than with "free" trophozoites. This method is convenient and successfully maintained the fastidious parasite.

Keywords: *Giardia intestinalis*, clonal growth, TYI-S-33, agarose, trophozoites

Studies on *Leishmania* infection of *Phlebotomus alexandri* the potential vector of Leishmaniasis by molecular techniques in Iran

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Objectives: Leishmaniasis is the most important and prevalent vector borne diseases in Iran. The present study was conducted on this species to determine its role in the transmission of *Leishmania* parasites in four western provinces.

Materials and Methods: Specimens were collected by sticky traps and CDC light traps and identified by valid morphological keys.

Results: *Phlebotomus alexandri* was found to be infected with *L. major*, *L. infantum*, *L. tropica* and *L. donovani*. The *L. donovani* and *L. tropica* infection of this sand fly species by using molecular methods is the first report in our country. Results showed that in Eastern Azerbaijan province this sandfly was infected by *L. infantum* and in Kermanshah Province it was infected *L. major*. In Fars province it was infected to all four mentioned *Leishmania* species but in Khuzestan province it was infected to *L. major* and *L. infantum*. It is concluded that, studies on the ecology, *Leishmania* infection rate and the control of this sand fly species should be revised in different leishmaniasis foci of Iran and special attention should be paid in the control of this species by health authorities.

Keywords: *Phlebotomus alexandri*, *Leishmania* species, Leishmaniasis, Iran,

Fascioliasis over the millennia: paleoparasitological evidences

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Fascioliasis is a parasitic disease caused by two trematode species, *Fasciola hepatica* with almost worldwide distribution, and *Fasciola gigantica* restricted to given regions of Africa and Asia. the absence of fasciolids in the Neotropical Region supports the origin of Fasciolidae after the fragmentation of Gondwanaland into Africa and South America occurred around 90–100 million years ago (mya). Paleoparasitological records showing the existence of this parasite after and before domestication in Europe, USA and Korea.

In Iran the oldest record of Fascioliasis back to Sassanid empire (224–651 CE). During our ongoing investigation of the animal paleo feces in Chehrabad archeological salt mine we retrieved *Fasciola hepatica* eggs from a Persian onager *Equus hemionus onager* feces. At the present time the given geographical region is not so highlighted in animal fascioliasis but still we witness the prevalence of animal fascioliasis among the herbivores, sheep, cattle, goat. Chehrabad salt mine archaeological site is located outside of the Fertile Crescent, namely eastward on the other side of the Zagros mountain chain and neighbouring the Guilan province located eastward from the Zanzan province. It has been located in the Fertile Crescent where *F. hepatica*, originally infecting wild ovicaprine in the mountainous areas, adapted to livestock throughout the domestication of sheep, goats and also cattle in that area. The finding of this parasite species in Chehrabad indicates that this fasciolid was already spreading eastward by this time.

***Cercopithifilaria*: a neglected but widespread dermal filarioid of dogs in Iran**

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Background and objectives: Canine filarioids presenting haematic circulating microfilariae (e.g., *Dirofilaria immitis*, *Dirofilaria repens* and *Acanthocheilonema reconditum*) are the better known worldwide compared to those presenting dermal microfilariae (e.g., *Onchocerca lupi* and *Cercopithifilaria* spp.). Filarioids belonging to the genus *Cercopithifilaria* are reported to infect dogs worldwide; indeed, *Cercopithifilaria baina*, *Cercopithifilaria grassii* and *Cercopithifilaria* sp. II sensu Otranto et al. 2013 have been reported from domestic dogs (*Canis lupus familiaris*) and red fox (*Vulpes vulpes*). Rhipicephalus sanguineus sensu lato is the competent biological vector of these nematodes. Given the lack of data about the infection of dogs in Iran with *Cercopithifilaria* spp. this study aimed to assess, for the first time, the presence of infection in this country.

Methodology: From October 2018 to June 2019, skin biopsies of inter-scapular region were collected from 489 dogs older than 6 months in Hamedan (n=103), Kermanshah (n=46), Khorram Abad (n=100), Yazd (n=80), Amol (n=80) and Ahvaz (n=80). All of the sampled dogs except for 7 were kept in shelters and breeding centers. Also one golden jackal (*Canis aureus*) in Hamedan was biopsied. Dermal microfilariae were detected by microscopic examination of skin snips which were soaked in saline solution at room temperature for over 6 hours. The microfilariae species were identified morphologically.

Results: Microfilariae of *Cercopithifilaria* spp. were found in skin biopsies of 26/489 (5.32%) dogs from Kermanshah (23.91% – 11/46), Yazd (8.75% – 7/80), Hamedan (4.85% – 5/103) and Amol (3.75% – 3/80). No infected dog was detected in Khorram Abad and Ahvaz. Mean length and width of microfilariae were 174.92 µm and 7.64 µm with a rounded head and a short dorsoventrally flattened body (n=21). Based on morphologic and morphometric characteristics of the microfilariae, they were identified as *C. baina* with a blunt caudal extremity. No infection was detected in examined client-owned dogs and red fox.

Discussion and Conclusion: This study provides first information about infection of dogs in Iran with *Cercopithifilaria* spp. Further investigations for possible presence of *C. grassii* and *Cercopithifilaria* sp. II in the carnivores of Iran besides PCR examinations followed by phylogenetic analyses of sequenced samples and identification of the parasite in ticks is suggested.

Keywords: *Cercopithifilaria baina*, Dermal microfilariae, Canine, Vector-borne, Neglected

Generation of a CRISPR/Cas9-based vector specific for gene manipulation in *Leishmania major*

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Background: Gene manipulation strategies including gene knockout and editing are becoming more sophisticated in terms of mechanism of action, efficacy and ease of use. In classical molecular methods of gene knockout, homologous arms are designed for induction of crossing over event in double strand DNA. Recently, CRISPR/Cas9 system has been emerged as a precise and powerful tool for gene targeting. In this effort, we aimed to generate a CRISPR/Cas9-based vector specific for targeting genes in *Leishmania* parasites.

Methods: U6 and DHFR promoters and neomycin-resistance gene were amplified from genome of *L. major* (MHRO/IR/75/ER) and pEGFP-N1, respectively. U6 promoter was cloned in pX330 vector which is named as pX330-U6. DHFR promoter and neo resistance gene sequence fragments were fused using a combination of SOE (Splicing by overlap extension)-PCR and T/A cloning techniques. To generate pX-leish, fused fragments subcloned into the pX330-U6. Two sgRNAs were designed to target the gp63 gene and cloned in pX-leish.

Results: The pX-leish vector was designed for simultaneous expression of cas9 and G418 resistance proteins along with a self-cleaving 2A peptide for efficient separation of the two proteins. In this study pX-leish was designed with 3 features: 1) Compatible promoters with *Leishmania* parasites. 2) Insertion of antibiotic selection marker 3) Designing an all-in-one vector containing all components required for CRISPR/Cas9 system.

Conclusion: This modified system would be valuable in genome manipulation studies in *Leishmania* for vaccine research in future.

Keywords: *Leishmania major*, CRISPR/Cas9, Gene manipulation, NHEJ

Experimental preservation of helminth eggs in different conditions over the time to illustrate the taphonomic changes in archeological contexts

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Introduction and objectives: Preservation condition of parasitic eggs obtained from ancient remains differs based on environmental factors. *Helminths* eggs retrieving from different environmental conditions were artificially experimented in the laboratory aiming to investigate taphonomic changes.

Materials and Methods: Five plates containing clay and one plate with saturated clay by NaCl with the mixture of helminth eggs were used in this study. Also 10 ml of normal saline were added to each plate and well mixed. Finally, each plate was placed in defined condition.

Results: Some helminth eggs were retrieved in defined conditions following rehydration of the samples. All the eggs were photographed and compared with original ones from morphological points of view.

Conclusion: Various factors such as temperature and humidity changes, affect the morphology of parasitic eggs over the time. Experimental studies can be helpful in understanding natural events.

Keywords: *Helminth* eggs, Morphological changes, Experimental study

Calmodulin-specific small interfering RNA induces consistent expression suppression and morphological changes in *Echinococcus granulosus*

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Background: Among parasitic helminths, biological features of *Echinococcus granulosus* have been a focus of particular interest in biology and medicine. The determinants and underlying molecular mechanisms of *Echinococcus* development in different host settings is largely unknown. The phenomenal bi-directional development of *E. granulosus* protoscoleces into multi-proglottid and/or microcysts, is a fascinating feature of the parasite cultivation. Calmodulin (CaM) is the major intracellular Ca²⁺ binding protein in plant and animal organisms. Many Ca²⁺-related processes in the physiology of eukaryotic organisms are CaM-dependent, however little is known on the role of CaM in platyhelminths growth and development.

Small interfering (si) RNA-induced manipulations of the genes involving in the parasite development is an opportunity to explore novel approaches for cystic Echinococcosis (CE) prevention and management. Regarding the fundamental role of CaM in cellular function of the parasites, in this study, we investigated the molecular and morphological changes induced by siRNA on CaM in different in vitro stages of *E. granulosus*.

Methodology: Three developmental stages of the tapeworm, protoscoleces, microcysts and strobilated worms, were cultivated in vitro in mono- and di-phasic media and three delivery methods, i.e. electroporation, soaking and electro-soaking, were used for RNA interference. The level of mRNA suppression as well as the phenotypic changes of the parasites were measured.

Principal Findings: Following RNA interference, EgCaM mRNA suppressions of 65-99% were recorded in different stages of the tapeworm as compared to untreated / unrelated siRNA controls. Silencing expression of EgCaM in different stages of *E. granulosus* resulted in reduced viability and body contractions and morphological changes.

Conclusions: Lower viability, growth retardation, morphological abnormalities as well as EgCaM expression suppression were documented in the parasite implying potential of siRNA technology for the prevention and management of CE.

Keywords: RNA interference; Calcium-binding protein; Strobilation.

***In vitro* effect of praziquantel on *Echinococcus granulosus* protoscoleces deterioration and expression of calcineurin phosphatase gene**

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Background: Praziquantel is a drug to treat a range of platyhelminth infections like hydatidosis. Its exact mechanism is not clear, but probably increases the permeability of cell membranes towards calcium ions. Calcineurin, a phosphatase protein enzyme, has an important role in calcium-signaling pathway. This study evaluated *in vitro* effect of praziquantel on *Echinococcus granulosus* protoscoleces deterioration and gene expression of calcineurin.

Methods: Genotype G1 protoscoleces of *E. granulosus* obtained from hepatic hydatid cysts of sheep. *In vitro* exposure to praziquantel at 250 μ M, 150 μ M and 50 μ M concentrations and time course of various performed and their viability microscopically determined as well as gene expression assessments of calcineurin subunits A, B as main genes and GAPDH, as reference gene, at concentrations

250 μ M and 50 μ M after 24h. Real-time PCR were performed and mRNA expression was assessed.

Results: Protoscoleces showed gradual morphological deterioration of evagination, ballooning, tegumental blebbing and hooks shedding. The lethal effect of praziquantel at 50 μ M, 150 μ M and 250 μ M, after 10 days, were 75%, 80%, and 100%, respectively. Both calcineurin subunits A and B showed significant up-regulation for both concentrations, with higher folding expression values of later gene.

Conclusion: Morphological deterioration of the protoscoleces were time dependent but visible from early hours of exposure. For the first time, the effectiveness of praziquantel on up-regulation of calcineurin in hydatid cyst protoscoleces demonstrated.

Keywords: Praziquantel, Calcineurin, *Echinococcus granulosus* protoscoleces, *In vitro*

Tracking of Lice Infestation over the Time in the World

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The history of lice and human is long. It is possible that when human ancestors had lived on the earth, there were lice infestation. The chimpanzees are closest to humans, and they also carry *Pediculus*. At least for 25 million years, primate lice are feeding them. About 4 million years ago, the ancestors of humans, and other primates have been in contact with lice. Low levels of health and communal life have led to the spread of these ectoparasites among humans. This could have caused epidemics of infectious diseases transmitted through this small bloodthirsty insect in various societies. One of the widely known of these microbial agents is *Rickettsia prowazekii* which causes typhus. Germs have been the cause of the deaths of millions of people throughout history, and their role cannot be ignored in human life from the past. Many samples of lice and nit lice from ancient times have been obtained today. Some of them come from mummies that are well kept and give us a lot of information. In fact, lice derived from mummies are the best protected parasites from ancient times. The other ways to find lice and nitlice from ancient times is the study of old combs sometimes found in archaeological excavations. A thorough examination of these combs in various researches has led to finding ancient lice. An accurate molecular study of the obtained lice and comparing it with current samples can provide us good information on how humans migrate on earth. In our research, we have found the oldest Iranian lice that will require further molecular studies in the future.

Keyword: Paleoparasitology, Lice, Mummy

Genotyping of *Fasciola hepatica* and *Fasciola gigantica* in Infected Domesticated Animals In Lorestan Province using Melting Analysis (HRM)

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Abstract: *Fasciola hepatica* and *Fasciola gigantica* are the cause of Fasciolosis, this disease is an important zoonosis disease in the world and affects livestock, especially sheep and cattle, which causes mortality and economic losses. It is difficult to make an exact morphological distinction between these two species. We used the High Resolution Melting analysis (HRM) method to differentiate the *F. hepatica* species from *F. gigantica* in order to use it to resolve the problems of distinguishing and differentiating these two species. The aim of this study was to differentiate between *Fasciola* species by using HRM in isolates taken from Lorestan province.

Material and method: In general, 80 adult liver flukes were collected from infected slaughtered animals including cattle, sheep and goats from Lorestan province. Genomic DNA was extracted using commercial DNA extraction kit. The multi locus sequences of mDNA including COX1, COX3 and ND6 were amplified employing real-time PCR & HRM analysis and using specific and universal primer pairs was design for identification between *Fasciola* spp.

Results: After the analysis it became clear that universal primers cannot be used to distinguish between these two species, but in the contrary, specific primer pairs of each species can be able to best differentiate between them. Molecular identification using specific primer pairs were consistent with morphological identification in all samples.

Conclusion: HRM is a simple, fast and reliable method for detecting and differentiating *F. hepatica* from *F. gigantica* and can be used for diagnostic and epidemiological purposes.

Keywords: *Fasciola hepatica*, *Fasciola gigantica*, Fasciolosis, PCR, HRM, COX1, COX3, ND6.

Assessment of apoptosis induction by *Holothurian leucospilota* in *Blastocystis hominis* isolated from human samples

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Background and aim: *Blastocystis hominis* is one of the most common intestinal protozoa which is found in large intestine in humans. This is the first in vitro study on the activity of methanol extracts of *Holothurian leucospilota* on the *Blastocystis hominis*.

Materials and methods: After reproduction of parasitic cells in RPMI 1640 medium, 100 µl of sea cucumber extracts with the different concentrations and 100 µl of cultures containing 1.5×10^5 cells were added to plates. These plates were incubated at 37 ° C and examined after 24, 48 and 72 hours and then the percentage of cell survival was determined of the using MTT method and result was reported as IC50 . To differentiate between apoptosis and necrosis, the inhibitory concentration of the extract was evaluated after 72 h using flow cytometry and DNA fragmentation tests.

Results: The value of IC50 for 24, 48 and 72 h were 219, 56 and 21 µg/ml respectively. The status of apoptotic cells was evaluated after 72 h exposure of the parasites to the extract with concentration of 21 µg / ml using flow cytometry and DNA fragmentation methods.

Conclusion: These findings demonstrate the potential of methanol extract of sea cucumber as a potent natural alternative of *Blastocystis hominis* for treatment of *Blastocystis* infection.

Keywords: *Blastocysts hominies*, Apoptosis , *Holothurian leucospilota*

Identification of *Fasciola* Species Isolates from West Azarbaijan, Iran, Based on ITS1 Sequence of Ribosomal DNA Using a PCR-RFLP Method

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Background: Fascioliasis is a common disease among humans and animals. It has global distribution and is developed by hepatic trematodes, *Fasciola hepatica* and *Fasciola gigantica*. The aim of this study is to identify *Fasciola* worms isolated from cattles and sheeps in West Azarbaijan, Iran, using PCR-RFLP.

Methods: Our subjects are eighty *Fasciola* worm samples (50 worms from cattle and 30 worms from sheep) were collected during abattoir inspection from livers of naturally infected cattles and sheeps. After DNA extraction, PCR was performed on region ITS1 of rDNA. Tsp509I digest restriction enzyme was selected for RFLP method that caused the separation specifically of *Fasciola* species.

Results: Using PCR method for the extracts resulted a 550-600 bp band (ITS1 fragment) which was similar in all subjects. The Tsp509I restriction endonuclease was used in RFLP method. RFLP produced a differential pattern including *Fasciola hepatica* and *Fasciola gigantica* so the results revealed that 9 out of 10 isolates were of *F.gigantica* type, whereas 1 isolate presented as *F.hepatica*. In the present study, *F. gigantica* and *F.hepatica* were coexisting in cattle and sheep.

Conclusion: The PCR-RFLP assay using Tsp509I restriction enzyme provides a simple, practical and economic method for identification of *Fasciola* parasites at the level of two species; *F.hepatica* and *F.gigantica*. Prevalence of *F.gigantica* in the province is so higher than *F.hepatica*.

Keywords: *Fasciola hepatica*, *Fasciola gigantica*, ITS1, PCR-RFLP, Iran.

Comparison of the hydatid cyst membrane permeability of Albendazole Sulfoxide in free nano form and loaded on magnetic solid lipid nanoparticles

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Background & Objective: Nowadays, Albendazole sulfoxide (ABZSO) is using alone or combine with other curative methods for the treatment of hydatid cyst. Since this routine method of drug therapy need to change in the formulation and drug delivery method regard to low drug solubility in the water, little intestinal absorption, systematic distribution, and side effects; current study aimed to comparison of the hydatid cyst membrane permeability of Albendazole Sulfoxide in free nano form and loaded on magnetic solid lipid.

Materials and Methods: Nanoemulsion using ultrasound was used to the synthesis of the Albendazole Sulfoxide in free nano form (ABZSO-NPs) and loaded on magnetic solid lipid nanoparticles (ABZSO-MSLNPs) in concentration of 2000 µg/ml and 2500 µg/ml. Physicochemical characterization of these nanodrugs, drug loading (DL %), and drug release were evaluated. Next, the permeability of mentioned nanodrugs in the hydatid cysts membrane and their efficacy were investigated by quantifying the drug concentrations in cyst fluid using high-performance liquid chromatography (HPLC).

Results: In the concentration of 2000 µg/ml of mentioned nanodrugs, the permeability of ABZSO-MSLNPs was higher than ABZSO-NPs (83.3±9.7 µg/ml vs. 44.7±10.7 µg/ml). Also, in the concentration of 2500 µg/ml of these nanodrugs, the permeability of ABZSO-MSLNPs was higher than ABZSO-NPs in concentration of 2000 µg/ml (148.9±31.7 µg/ml vs. 88.9±3.2 µg/ml). In addition, the permeability of theses nanodrugs was more prominent in the smaller and fertile hydatid cysts.

Conclusions: According to the higher permeability of ABZSO-MSLNPs, this nanodrug can use in the magnetic drug delivery system for better treatment of hydatidosis.

Keywords: Hydatid Cyst; Albendazole Sulfoxide; SLNs; Permeability; HPLC.

The need for biobanking of parasites: experiences from the Iranian Registry of Hydatid Disease

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Introduction and aims: A variety of biospecimens can be collected in biobanks to diagnose diseases, monitor response to treatment and help in the development and testing of new therapies within the healthcare system or for research purposes. Running National Registry of Hydatid Disease program along with the corresponding collection of biological samples in the form of an integrated biobank could improve our understanding of natural history of Echinococcosis.

Materials and methods: Iranian registry of hydatid disease was established in 2013 by the Research Center for Hydatid Disease in Iran at Afzalipour Medical Center in Kerman. Systematic biobanking of hydatid-related specimens is an important part of the registry. For achievement of this purpose operational, technical and ethical roles were prepared as standard operating procedures (SOPs). Also patient information, clinical data and cyst characteristics were recorded in a specially-designed software. Four different biological specimens including cyst layers, protoscoleces, serum and whole blood were entitled in the biobank. Two-dimensional barcoding system as well as -70 °C storage system were used.

Results: Our experiences indicate that establishing integrated biobank along with the parasitic disease registry programs provide high quality biological resources with associated data for retrospective, comparative genetic/genomic investigations.

Conclusion: Parasite biobanks with a potential to extend as a national-scale biobanks provide high added values throughout the country for the benefit of patients, students as well as the academies and research institutes. Similar successful biobanks have been developed in countries e.g. Switzerland, Tunisia, China and Europe.

Key words : Parasite Biobanks, Echinococcosis, Hydatid, parasite, Registry

Knowledge, attitudes, and practices of students regarding Pediculosis capitis in the city of Corum in Turkey

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Objectives: Pediculosis capitis is a widespread public health problem in many developing and developed countries, including Turkey. The education of present and future parents regarding the biology and control of head lice is of paramount importance for an effective control. The aim of this study was to assess the knowledge, attitudes and behavioural practices of university students as future parents regarding head lice.

Materials and Methods: The study was performed in the Hitit University, Faculty of Health Sciences. The sample group consisted of 265 randomly selected students, aged 17-25 years, from three departments.

Results: Overall, 217 (81.9%) of the participants stated that they had some preliminary information about head lice, while 117 (44.2%) of the students mentioned that they were infested one or more times with head lice during their childhood. Significantly more female students were infested in the past (55.7%) than male students (20.3%), and 83% of all students knew that pediculosis is more prevalent in girls than boys. The majority of the participants wrongly thought that lice could jump from one person to another (86.4%); if a family member is infested with lice, the whole family should be treated (78.9%); if nits are present on the hair, the person was also infested with living lice (78.5%); lice can survive for several days outside the human body (77.4%); head lice could also infest dogs, cats and birds (76.6%); regular combs, brushes, hats, scarfs, ribbons and similar objects are important source of infestation (92.1%); lice feed on dandruff and sweat (74.0%); head lice are vectors of diseases (56.2%); and the environment of the infested person should also be treated for lice (68.7%). On the other side, the majority of the students correctly thought that lice feed only on blood (79.6%); all developmental stages are blood-feeders (64.2%); direct contact with infested individuals is the usual way of getting lice (91.7%); infested individuals should be treated with pediculicides (75.1%); and not with home remedies (63.8%). Most of the students (67.2%) stated that they saw living lice and 55.5% saw lice eggs, while 55.8% believed that they did not have enough information to distinguish between life eggs and nits.

Discussion and conclusion: The knowledge of future parents in the city of Corum on head lice biology, prevention and treatment is limited. While the majority of students answered correctly some of the questions, the proportion of wrong answers to most of the questions was high. Accordingly, it is recommended that in order to reduce the incidence of head lice in the community, students who in majority are future parents of children, should be better educated on the biology and control of head lice.

Keywords: head lice, pediculosis, knowledge

Introducing naltrexone and propranolol as new adjuvants for protozoan vaccines

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Introduction: Development of human vaccine for intracellular parasitic protozoa which immunity against them relies mostly on activation of cellular immunity or Th₁ responses, has been always entangled with lack of suitable adjuvant. The most applied adjuvant for designing human vaccines is alum or aluminium hydroxide that shifts immunity toward Th₂ responses and humoral immunity. Since 10 years ago, naltrexone and propranolol have been used successfully as adjuvants for this kind of vaccines in Urmia School of Medicine with the advantage that they are already FDA approved drugs.

Methods: These adjuvants, alone or along with alum were used in immunizing mice against different kinds of antigens from *Leishmania major*, *Toxoplasma gondii* and *Plasmodium berghei* and their potency of enhancing specific cellular immunity was assessed by measuring IgG₁ and IgG_{2a}, spleen cells proliferation, cytokine production, delayed type hypersensitivity, and challenging with live parasites.

Results: In almost all of the studies, a remarkable increase in immunity and survival of immunized mice with these adjuvants were seen. In case of *L. major* which immunity against it needs Th₁ responses, naltrexone alone showed a better effect in comparison with when it was used along with alum, but immunizing mice with antigens of *P. berghei* and *T. gondii* along with alum-naltrexone or alum-propranolol revealed the best protective effects.

Discussion: Both naltrexone and propranolol shown that they are potentially effective adjuvants and are able to strengthen the cellular immunity against intracellular protozoa and it deserves to put more efforts to discover their complete abilities, so that they might be used in immunizing humans in the future.

Key words: Vaccine, Immunization, Parasitic Protozoa, Adjuvant, Naltrexone, Propranolol

Oxidative and Nitrosative Stress in Protozoal Infections

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Background: During an immune response, the generation of oxidants may take place via different routes. Parasites themselves can also be directly responsible for oxidant release through degradation products of their own metabolism.

While useful in immune protection, non-targeting toxic oxidants have potentially important negative side-effects. The free radicals can inflict direct damage to macromolecules, such as lipids, nucleic acids, and proteins. Accumulation of oxidative damage may eventually lead to degenerative pathologies and shortened lifespan. Organisms minimize these damaging effects through antioxidant defenses, which comprise a store of endo and exogenous compounds that inhibit oxidant chain reactions by directly neutralizing the oxidants. The harmful effect of free reactive oxygen species (ROS) and reactive nitrogen species (RNS) radicals is termed oxidative stress and nitrosative stress, respectively.

Keywords: Oxidative Stress, Nitrosative Stress, Antioxidant, Protozoa

Novel approaches for vector control

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Objectives: There are several vector borne diseases in the world including Malaria, different types of Leishmaniasis, dengue fever, Chikengunya, yellow fever, West Nile virus, Rift valley fever, Zika, river blindness, Chagas disease, Crimean Congo Hemorrhagic Fever (CCHF), relapsing fever, Lyme disease, lymphatic filariasis, Japanese encephalitis, sleeping sickness, plague, schistosomiasis, O'nyong nyong virus, Mayaro virus. They become epidemic and effect on the population health.

Materials and Methods: The data was collected from different published paper as well as WHO documents.

Results: There are several vector control in this situation such as environmental management, Health education, indoor residual spraying, vaccination, space spraying, using impregnated bednet, larviciding, light traps, repellents, biological control, blanket impregnated, male sterility, cattle dipping, attractive sugar baits, Wolbachia, paint in, dog collar, chemosterilants, anticoagulants, wall netting, live traps, dead traps.

Discussion: The importance of novel approaches for vector control in current situation will be discussed.

Keywords: Vector Borne diseases, vector control, World

Establishment of Afghanistan Registry of Cystic Echinococcosis System: the first prospective registry with an international future

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Background: Cystic Echinococcosis (CE), a worldwide zoonosis, is endemic in some parts of the world. Unfortunately the actual prevalence of the disease is unknown in Afghanistan due to the lack of efficient reporting systems designed to take into account the particular features of the disease. Neglect of CE makes diagnosis and clinical management difficult outside referral centres, with inconsistencies in clinical practice and often unnecessary procedures carried out that have associated risks and costs. The Afghanistan registry of CE (ARCE) is a prospective centre registry of CE patients seen from 2019 in Afghan health centres; data are voluntarily submitted to the registry. Its aims are to show the prevalence of CE in Afghanistan, bring the importance of this infection to the attention of health authorities, encourage public health policies towards its control, and stimulate biological, epidemiological and clinical research on CE. Fortunately, the Iranian National Center for Registry of Cystic Echinococcosis assist us very much and from January 2019 to now, a total 11 patients were enrolled in 2 centres, 5, 5 and 1 hydatid cyst from brain, liver and lung. We discuss preliminary data and challenges of the ARCE, a center under umbrella of the European registry of CE, which has been implemented within the Seventh Framework Programme project HERACLES (Human cystic Echinococcosis ReseArch in Central and Eastern Societies) since September 2014.

Hydatid cyst as a major health problem in endemic regions

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Hydatid diseases is a major health and economic issue in vast areas of the world. This zoonotic disease is endemic in the countries which deal large in agriculture and husbandry such as Mediterranean countries, south Africa, the Middle East, south America and New Zealand. In these countries aside from economic losses to livestock industry, involvement of human being with this parasite is a major health problem.

The worm (*Echinococcus granulosus*) has two hosts, the definitive host is certain carnivores notably dogs and mature parasites live in their intestine, and intermediate host, that includes herbivores such as sheep. Human being is infected by consuming the eggs of *Echinococcus* along with food or water polluted with dog feces.

In human intestine embryo is released from eggs, and is transported by the blood to almost all tissues in the body (except for a few organs such as cornea) where they transform to cystic form. Liver and lungs are the first and second organs in chance of being infected with parasite. Natural history of hydatid cyst in involved organs is relentless growth until complications occur, unless diagnosis is made before complications.

The most effective treatment for this disease is surgery, although other modalities such as percutaneous drainage and medical treatment with benzimidazole derivatives are used in selective cases. It must be remembered that diagnosis and specially treatment isn't straightforward every time and complication associated with treatment may be challenging for clinicians. In this paper clinical presentation, diagnostic challenges and treatment related morbidity and mortality will be discussed.

Poster

A Survey of Gastro-Intestinal Parasites of Pigeons (*Columba livia*) in Ahvaz, Iran

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Objectives: Pigeons are found in almost every part of the world. However, despite the widespread distribution and importance of these birds, there is little information about their contamination with GI worm parasites. By identifying GI parasites and examining their evolution, they can prevent the spread of contamination through proper prevention and treatment.

Materials & Methods: 50 pigeons from the bird shops of Ahvaz city in summer of 2018 were made. After the post-mortem examination the abdominal and thoracic cavity were opened, followed by systemic autopsy examination which include, the oesophagus to cloaca. The contents were examined under stereomicroscope and all helminthes were fixed in 70% ethanol for further identification. All parasites were identified using the key Soulsby (1986) and Yamaguti (1961).

Results & Conclusion: The small intestine was the only part of the gastrointestinal tract that was infects. A total of 50 pigeons, 4 % (n=2) and 12 % (n=6) were infected with nematodes and cestodes respectively. The results showed that among the infected samples, 2 cases of *Ascaridia colomba* (4%), 3 cases of *Raillietina acinobutrida* (6%) and 3 cases of *Raillietina cysticula* (6%) were observed. Trematode infection was not found among infected pigeons. Due to the presence of infections in the pigeons of Ahvaz city and the cross-linking of this bird with other birds, it can be concluded that this bird plays a very important role in infecting other birds and even native poultry as well as industrial poultry on these parasites.

Keywords: gastrointestinal parasites, pigeons, Ahvaz.

Identification of *Leishmania* Species with High Resolution Melting PCR to Distinguish Exact Parasite Responsible in Cutaneous Leishmaniasis in Areas Where the Disease Newly Appeared in North East Iran

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Background and Aim: Cutaneous leishmaniasis is a zoonotic disease endemic in nearly 90 countries including Iran with estimated annual incidence of 1.5 million. Microscopy of smears taken from lesions is the most common and standard test for identification of the parasite. However, performing different techniques resulted in doubts to correctly report distinguishable parasite species. Examining new reliable molecular techniques therefore, still are sought in practice.

Methods: This cross sectional study was conducted in four villages in Sabzevar, northeast Iran, where leishmaniasis is newly appeared in 2016. Diagnosis of leishmaniasis was diagnosed in 50 Giemsa staining slides prepared from skin lesions of different parts of the body including hands, face, legs, and ears. All Giemsa stained slides underwent microscopy. DNA extraction from Giemsa stained slide was used to identify the sex and species of the *leishmania* parasites by high resolution melting polymerase chain reaction (HRM-PCR) method. Data were analyzed using SPSS software.

Results: We found that 100% samples in four villages (Bagherabad, Khosroabad, Chobin Chesham) were *Leishmania major*. However, in contrast to the most recent studies which reported that only *L. major* is responsible parasite, we found that, for the first time, in Bagherabad 4 cases out of 13 samples were *L. tropica*.

Conclusion and Discussion: Our results suggest that utilizing of new advanced molecular technique such as HRM-PCR should perform to correctly identify exact species present in the given regions. This would be the first necessary step to control and combat the disease

Keywords: Cutaneous leishmaniasis, high-resolution melting Polymerase Chain Reaction, *Leishmania major*, *Leishmania tropica*

Clinical Symptoms and Laboratory Signs of Bronchopulmonary Infections Due to *Lophomonas blattarum*

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Introduction: *Lophomonas blattarum* is an emerging important cause of bronchopulmonary infections. According to previous studies in our region and high prevalence of this infection, we decided to review clinical symptoms and laboratory signs of patients with *Lophomonas blattarum* who were admitted in Imam Reza and Dr. Sheikh hospitals located in Mashhad, Iran.

Materials and Methods: In this cross sectional study, we reviewed the records of patients with *Lophomonas blattarum*, who were admitted in different wards of Imam Reza and Dr. Sheikh hospitals in 2014-2016. The checklist and required information were provided for each patient and then were analyzed by using chi-square and Fisher's Exact Test's.

Results: The most common clinical symptoms in adults were, fever (35%), weakness (27.5%), weight loss (27.5%), most respiratory symptoms included coughing (55%), dyspnea (50%), hemoptysis (20%), the most prevalent predisposing of Lophomoniasis was cancer (27.5%), most laboratory signs : increased neutrophil count (67.5%) and lymphocyte reduction (62%). The main symptoms in children were, fever (35.1%), most respiratory signs and symptoms: coughing (85.7%), dyspnea (37.7%), most laboratory symptoms: lymphocyte reduction (39%) and eosinophilia (19.5%).

Conclusion: We concluded the most common clinical signs and symptoms and laboratory signs among adults and children were fever, coughing, dyspnea, lymphocyte reduction and eosinophilia.

Keywords: Broncho Alveolar Lavage (BAL), *Lophomonas blattarum*, Pulmonary Infection.

The Prevalence of *Lophomonas Blattarum* in Asthmatic Patients

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Introduction: *Lophomonas blattarum* (L.b) is a parasitic protozoa which is important in respiratory patients. It is transmitted through inhalation, and it is a potential element in bronchial and respiratory infections among the patients with pneumonia and asthma. The purpose of this study was the investigation of *Lophomonas blattarum* frequency in asthmatic patients and control group.

Materials and Methods: Participant in this cross-sectional study was patients who referred to respiratory specialist clinic of Imam Reza Hospital. Direct smear of samples of patient's phlegm and giemsa stained smear were prepared and observed under light microscope and this was done for control group as well. Then the prevalence of the parasite is measured by investigating the samples.

Results: There were 4(10%) positive samples among 40 asthmatic participants. 3 women and 1 man with average age of 43years. All four patients were controlled regarding the severity of the disease. None of the control group members was positive for (L.b). 10% of asthmatic patients were infected with *Lophomonas blattarum* whose symptoms eliminated after treatment with metronidazole.

Conclusion: It is therefore suggested to test asthmatic patients for *Lophomonas blattarum* who suffer from respiratory symptoms despite the proper control of their disease.

Keywords: *Lophomonas blattarum*, Asthma, Mashhad.

Prevalence of Intestinal Parasitic Infections in Kidney Transplant Recipients

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Introduction: Over the last two decades protozoa are one of the most common causes of infectious diseases in people with impaired immune system like renal transplant recipients. The aim of this study has been to determine the prevalence of intestinal parasitic infections in renal transplant recipients of Mashhad, Iran.

Materials and Methods: This descriptive study was conducted from January 23, 2015 to January 18, 2016 on renal transplant recipients of Mashhad. Three stool specimens were collected and examined by four techniques: wet mount, formalin-ethyl Acetate concentration, Ziehl-Neelson staining and trichrome staining.

Results: Of 71 renal transplant recipients, 41(57.7%) were male and 30(42.3%) were female with the average age 39.5. The results of this research showed that 45.07% of renal transplant recipients and 2.81% of control group were infected with intestinal parasites. The parasites detected among renal transplant recipients included 20(28.2%) *Entromonas hominis*, 11(15.5%) *Giardia lamblia*, 3(4.2%) *Blastocystis hominis*, 2(2.8%) *Cyclospora cayetanensis*. The intestinal parasitic infections in control group were 1(1.4%) *Giardia lamblia* and 1(1.4%) *Blastocystis hominis*. In statistical analysis results of prevalence intestinal parasitic infections, there was a significant difference between the renal transplant recipients and control group ($P < 0.001$).

Conclusion: This study indicated that intestinal parasitic infections have relatively high prevalence in renal transplant recipients of Mashhad. So these patients should be tested periodically to prevent morbidity and mortality from parasitic infections.

Keywords: Intestinal Parasitic Infections, Renal Transplant.

Seroepidemiology of Canine Visceral Leishmaniosis in Semnan & Mahdishahr

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Abstract

The Mediterranean form of visceral leishmaniasis (VL), or kala-azar, is a potentially fatal vector-borne zoonotic disease caused by *Leishmania infantum* which is sporadic in most parts of Iran, while it is considered endemic in other parts. Domestic dogs (*Canis familiaris*) are the main reservoir hosts for human VL. The objective of the present study was to determine the seroprevalence of VL in the owner's dogs of Semnan and Mahdishahr rural area. For this purpose, 140 owner dogs with no clinical signs were randomly assigned to blood samples. Blood samples were tested by direct agglutination test (DAT) to detect the anti-*Leishmania* antibodies in dogs, using a cut-off value of $\geq 1:80$. we considered anti-*leishmania* antibodies titers at $\geq 1:320$ with a clinical sign as *Leishmania* infection and at $\geq 1:80$ with no clinical symptoms as parasitologically infected. Pathological specimens including spleen, liver and lymph nodes from an infected dog (1:20480) were prepared for Direct smears in Laboratory and staining with Giemsa. The stages of amastigote leishmania were observed in isolated tissues. The anti-*Leishmania* antibody ($\geq 1:320$) was detected in 4 dogs (2.9 %) of the total 140 studied dogs. No significant difference was found between VL infection and gender & age. The result of this study showed that VL with low endemicity is circulating in dogs of Semnan and Mahdishahr.

Key word: Visceral Leishmaniasis, Direct Agglutination Test, Dog, Semnan, Mahdishahr.

Genotyping of *Echinococcus granulosus* Isolates from Human in Khorasan Province, North-Eastern Iran

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Background: Human hydatidosis is endemic in northeastern Iran. The present study aimed to investigate molecular diversity of *Echinococcus granulosus* isolates collected from human surgically.

Methods: Sixty human hydatid cysts (58 lung cysts and 2 liver cysts) were collected through surgery from Ghaem and Emam Reza hospitals in Mashhad University of Medical Sciences during 2015-2016. Cysts were characterized using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the internal transcribed spacer 1 (ITS1) gene and sequencing fragments of the genes coding for mitochondrial cytochrome c oxidase subunit 1 (cox1) and NADH dehydrogenase subunit I (nad1).

Results: Overall, 55 out of 60 *Echinococcus granulosus* cysts (91.6%) were determined as the G1 strain, 4 cases (6.6%) were determined as the G6 strain and 1 sample was not identified.

Conclusion: Although sheep strain (G1) is dominated in human patients in Great Khorasan, the prevention of camel-dog cycle should pay attention in this region.

A Survey on the Influence of Toxoplasmosis on Some Reproductive Parameters in Male Rats

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Introduction: *Toxoplasma gondii* is one of the microorganisms that can contribute to male genital tract infection

Material and Methods: In this experimental study, 80 Wistar male rats were divided into control and infected. In the infected group, tachyzoites of *Toxoplasma gondii* RH strain and in the control group, normal saline was injected intraperitoneally. Then, every 10 days from day 10 to 80, fertility parameters including morphology, mobility, number and survival of sperm, serum LH, FSH, testosterone and histomorphometric characteristics of the testes were evaluated.

Results: Sperm motility during 20-70 days after inoculation, number of sperms on days 20, 40, 50, 60 and 70, natural morphology of sperm on days 20 and 70 and viability of sperm on days 20, 30, 60 and 70 was significantly decreased in the infected group compared with the control group. The amount of LH in the 20 to 70 study day decreased. The level of FSH and testosterone hormone was higher in the infected group versus the control group across all days of the study. In the histomorphometric studies of testicular tissue, the number of Leydig cells decreased only in the 20th day of the study; Sertoli cells decreased on day 60 and the number of spermatid layers decreased on day 40 in the infected group compared with control group. The epithelial thickness of the seminiferous tubes in the infected group was higher on days 40 and 60.

Conclusion: In this study, *Toxoplasma gondii* infection was found temporarily to interfere with the male reproductive system.

Keywords: *Toxoplasma gondii*, Rat, Infertility, sperm.

Comparison of the Effects of *Lendula Angustifolia* and *Coronus Moss* on Protoscoleces of Hydatid Cyst, in Vitro

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Objective: Echinococcosis (hydatid disease) is a dangerous zoonotic parasitic disease. One of the major problems associated with this disease is destruction of protoscolex production to prevent recurrence of secondary cysts. A variety of chemical substances with various adverse effects have been used to prevent secondary echinococcosis. The purpose of this study was to investigate the effects of hydroalcoholic extract of cranberry and lavandula on hydatid cyst protoscolices.

Material and Methods: Sheep livers infected with hydatid cysts were collected from slaughterhouses and transported to the parasitology laboratory of Hamedan University of Medical Sciences under appropriate cold chain conditions. The fluid containing protoscolex cysts was isolated under strictly sterile conditions with 70% Isopropyl alcohol, and sterilized gas and syringe, and collected into falcon 50mL conical centrifuge tube. Finally, liquid centrifugation was performed at 2000 g for 5 minutes. Afterwards, the supernatant was completely discarded and the precipitate containing live protoscolices were added onto the ROML 1640 medium and refrigerated until the test day. The sediment containing protoscolices were was colored using methylene blue as an indicator. Then, a drop of precipitated was observed and examined by optical microscopes to determine the viability of cells.

Results: the findings of this study show that cranberry and lavandula killed 87% and 94% of all activated protoscolices after 60 min at concentration of 100 µg/ml. Given various dosing intervals and concentrations, these treatments were most effective after 60 min at concentration of 100 µg/ml.

Conclusion: the current study revealed that cranberry and lavandula killed 87% and 94% of all activated protoscolices at concentration of 100 µg/ml. Thus, these herbs were shown to be powerful natural protoscolices killers. However, further research is required to accurately investigate the effects of cranberry and lavandula on human body.

Keywords: cranberry, lavandula, protoscolex, hydatid cyst.

A randomized, double - blinded clinical trial of the efficacy of permethrin 1% in shampoo for the treatment of head louse infestation in female primary schools in Ahvaz city, Iran

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Objectives: Population-based studies show that the prevalence rate of *Pediculosis capitis* is 0.9% to 20.5% in different areas of Iran. This study was done to evaluate the effect of the permethrin 1% on pediculosis of female primary school students in Ahvaz City (2015-2016).

Materials and Methods: Two applications of permethrin 1% were done, seven days apart. Efficacy of shampoo was studied with 2 days, one week and 2 weeks intervals. Outcome measure was defined as cure (absence of vital head lice/nit) after first application and before and after second applications. Data analysis was done using SPSS software. Differences between the groups and efficacy were tested using the chi squared test.

Results: One hundred eleven louse-infested schoolgirls were randomly treated with the shampoo permethrin 1%. One hundred eleven infected schoolgirls were treated with placebo shampoo. Permethrin shampoo produced a 56.8% recovery and ordinary shampoo (placebo) a 10.8% recovery, respectively, after 2 days post – treatment. Moreover, there was the significant difference between percentage recoveries of two treatment shampoos. Cure rates were in the permethrin group at days 7 and 14 days, respectively, 69.4% and 90.1%. Cure rates were in the placebo group at days 7 and 14 days, respectively, 18% and 18%. Meanwhile, the recoveries between two shampoos in 1 week and 2 weeks after treatments were significant. The highest percentage of recovery was observed in grade 3 children.

Conclusions: Pediculicide shampoos are effective, but education and modification of sociocultural factors are also significant.

Keywords: Permethrin Shampoo, Pediculicide, Head Lice, Primary School Girls, Treatment, Iran.

Study and identification of *Demodex* ectoparasite and its association with skin lesions among women of Ardabil, Iran

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Abstract

Objectives: *Demodex* is a common mite and ectoparasite in humans and animals. The establishment of *Demodex folliculorum* and *Demodex brevis* in human skin may cause some skin lesions, such as acne, rosacea and dermatitis. The aim of this study was to identify permanent *Demodex* ectoparasite in Ardebil women and its possible association with skin lesions.

Methods: Fifty women with dermal lesions (case group) and 50 women without dermal lesions (control group) were selected and underwent clinical and laboratory tests. After obtaining permission from the Ethics Committee and letter of satisfaction from the volunteers, demographic data and skin surface cut were prepared. Using a 10% KOH and lactophenol solutions, the samples were examined by microscopy. And based on valid diagnostic keys, *Demodex* was detected.

Results: Twenty-two out of 50 patients (with acne, rosacea, dermatitis and eczema) were contaminated with *Demodex folliculorum* (44%). And the highest levels of contamination were observed in women aged 20-30 years (22%). Of the 50 control subjects, *Demodex* contamination was confirmed in 10 cases (20%).

Conclusion: Based on the results of this study, it can be concluded that the rate of *Demodex* in patients with skin lesions is much higher than healthy subjects. Also, statistical tests confirm the significant relationship between *Demodex* and skin lesions (sig. = 0.023, $p < 0.05$). It is suggested that *Demodex* treatment should be considered in the therapeutic strategy of skin lesions.

Keywords: Ectoparasite, *Demodex*, Women, Skin lesions

Is Urine a Reliable Clinical Sample for the Diagnosis of Human Visceral leishmaniasis? A Systematic Review and Meta-Analysis

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Abstract

Aim: Visualization of amastigotes in lymph nodes, bone marrow, and other tissues samples remains the gold standard method for the diagnosis of visceral leishmaniasis (VL). This gold standard diagnostic method uses a technically challenging microscopy procedure that is often not accessible in many places in the world where VL is endemic. We report the current systematic review and meta-analysis to evaluate whether urine is a reliable clinical sample for diagnosis of VL.

Methods: Data were extracted from ten available databases during the period from 2002 to 2017. Overall, 29 articles fulfilled the inclusion criteria and were used for data extraction in this systematic review.

Results: Most studies (72.4%) using urine specimens were reported from five countries: India 6 (20.7%), Iran 5 (17.2%), Bangladesh 4 (13.8%), Japan 3 (10.3%) and Spain 3 (10.3%), respectively. The most common diagnostic tests performed on urine were Katex (62.1%), ELISA (24.1%), and the rK39 (17.2%) assays.

Conclusion: In meta-analysis the sensitivity and specificity of the three most commonly used diagnostic assays were rK39 (97%; CI: 91–99; 98%; 76–100), ELISA (91%; 82–95; 99%; CI: 94–100), and Katex (83%; 73–90; 98%; 98–100), suggesting that the rK39 assay provided the highest sensitivity and the ELISA assay provided the highest specificity for diagnosis of VL from urine samples. Our findings suggest that urine is a valuable clinical sample for the diagnosis of VL, particularly in areas where the gold standard test for VL is not available.

Keywords: Visceral leishmaniasis, Urine, Immunologic tools, PCR, Systematic review

Global Epidemiological Aspects of Feline Visceral Leishmaniasis: a Systematic Review and Meta-Analysis

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Abstract

Aim: Visceral leishmaniasis (VL) is a serious public health problem and cats, as secondary reservoir, have become an important source of information regarding the disease. Because most infected cats are resistant and remain asymptomatic in recent years feline visceral leishmaniasis (FVL) has been studied more than ever before in various parts of the world. The main objectives of this study investigated the status of FVL in the world and introduce better laboratory methods for more accurate diagnosis.

Methods: Data were extracted from ten available databases over the period of 1982 to 2017. Overall, 78 articles fulfilled the inclusion criteria and were used for data extraction in this systematic review.

Results: The overall prevalence of FVL with both serological and molecular methods was estimated 10% (95% CI: 8%-14%). In Italy, both the prevalence by seropositivity (24 %) and PCR positivity (21 %) was found to be higher than other countries. The most common used laboratory test was the indirect fluorescent antibody test (IFAT) (38%). Study on mixed type of cats was higher than other types and the most common parasite species was *L.infantum*(63%).

Conclusion: Our data showed the most accurate serological test for diagnosis of feline leishmanial infection is WB particularly in endemic areas. Our data shows the enzootic stability situation of FVL in European countries and will pose a risk for inhabitants there. Control of cat populations is recommended to reduce the transmission of disease among human populations.

Keywords: Feline visceral leishmaniasis, PCR, IFAT, Systematic review

***Toxoplasma gondii*: Oxidative Stress in Reproductive System in Experimentally Infected Male Rats**

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Abstract

Background: Toxoplasmosis as an important zoonotic disease caused by an obligatory intracellular parasite *Toxoplasma gondii*. Toxoplasmosis as a common parasitic disease have adverse effect on male and female reproductive system which can result in idiopathic impairment in male fertility. This study aimed to determine the testis stress oxidative status related to Toxoplasmosis in experimentally infected rats.

Material and methods: Eighty male Wistar rats were randomly divided to control and test groups. Animals in test group were inoculated by 10⁷ *T. gondii* RH strain tachyzoites and control group were injected by 0.2 ml of PBS at the same time. Both groups were following every 10 days until day 80 post inoculation. Oxidative stress markers including antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and non-enzymatic markers including Malondialdehyde (MDA), total antioxidant capacity (TAC) and Glutathione, were assessed based on commercial assay kits protocol (kiazist).

Results: Results showed that, the testis concentration of MDA level was significantly elevated after 70 (P<0.05) PI. The testis GSH level was also remarkably decreased after day 80 PI (P<0.05). In addition, the concentration of TAC testis was significantly decreased on day 80 70 (P<0.05) PI. Also, marked decrease of testis SOD activity was observed on day 80 (P<0.05) PI. The activity of CAT in testis tissue was significantly reduced after 60 (P<0.05) PI.

Conclusion: Toxoplasmosis caused oxidative stress in testis, so, it had adverse effects on the reproductive tissue (testis), which may cause temporary impairment infertility in male rats.

Keywords: *Toxoplasma gondii*, infertility, Rat.

Detection and Molecular Genotyping of Endosymbiont *Wolbachia* sp. Isolated from *Dirofilaria Immitis* in Northwest Iran

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ABSTRACT

Background: *Wolbachia* spp. are obligate, intracytoplasmic bacterial endosymbiont in nematodes and arthropods. In this study, we aimed to identify the molecular pattern of *Wolbachia* spp. of *Dirofilaria* (*D.*) *immitis* isolated from Northwest Iran.

Methods: Adult filarial nematodes were collected from domestic dogs and total genomic DNA was extracted. The purified DNA was used to determine the molecular pattern of the *Wolbachia* surface protein gene (WSP) sequence by PCR.

Results: Phylogeny and homology studies showed high consistency of the gene with previously-registered sequences for *D. immitis*. In fact, comparison of DNA sequences revealed no nucleotide variation between them. However, *Wolbachia* species sequences in *D. immitis* were significantly different from those of other *Dirofilaria* and filarial species. The greatest distance between WSP nucleotides of *Wolbachia* species was between *D. immitis* and those isolated from *Onchocerca lupi*, which appeared to be approximately 33 nucleotides.

Conclusion: Thus, perhaps certain *Wolbachia* strains affect the epidemiology of this vector-borne filarial disease. Our findings indicate that PCR could be a simple but suitable method to detect *Wolbachia* species. The results of this phylogenetic analysis and molecular characterization could aid in identifying *Wolbachia* and understanding their evolution.

Keywords: *Wolbachia*, *Dirofilaria immitis*, WSP, Iran, Dog

In vitro effect of some Iranian medicinal plants on the *Histomonas meleagridis* parasite

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Objective: This study aimed to investigate the in vitro effect of the extracts of five Iranian medicinal herbs on *Histomonas meleagridis* protozoan parasite.

Methods: This study was conducted in the following three phases in Khorramabad, Iran, in 2016: 1) preparing and extracting the 5 medicinal herbs (*Dracocephalumkotschyi*, *Saturejakhuzestanica*, *Pulicariagnaphalodes*, *Oleaeuropaea*, and *Echinophoraplatyloba*DC; 2) culturing the positive cases of fecal samples in the fortified commercial medium RPMI1640 up to 1×10^6 parasite/ml of the parasite; and 3) investigating the effect of 1.25, 2.5, and 5 mg/ml densities of each extract and metronidazole compared with the negative control on hours 2, 4, 6, 24, and 72 with *Trypan blue* vital stain.

Results: In this study, the *D.kotschyi* extract which destroyed 80% of the parasites in the first two hours was the most, and the *S.khuzestanica* extract was the second effective extract. However, metronidazole destroyed 60% of the parasites in the first two hours and 100% in the third day and may be excreted before being effective, showing relative resistance.

Conclusion: The results of the present study demonstrated that herbal medicines may be a natural source for the production of new agent to treat histomoniasis infection.

Therapeutic Effect of Hydatid Cyst Fluid and BCG on Inhibition of Melanoma Cancer Growth in C57 Black Mice

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Background: Cancer is the main cause of death in the developed countries(1). There are some scientific evidences indicating that parasitic infections induce antitumor activity against certain types of cancers(2, 3). Hydatid cyst is the larval stage of *Echinococcus granulosus*, that causes hydatidosis in human and livestock(4, 5). In this study, the effects of hydatid cyst fluid and BCG on Melanoma tumor (4, 6-9) in C57/black mice were investigated. In this study, therapeutic effects of hydatid cyst fluid and BCG vaccine on C57/black mice that had already been challenged with melanoma tumor were investigated.

Materials and methods: In this study 3 groups of mice were subcutaneously inoculated with melanoma cancer cells (line B16F10) in PBS inside their chest site. After 1 week, case groups were injected with hydatid cyst fluid, and BCG. And control group was left intact. Tumor size in the cases and control groups were measured and the data were analyzed using SPSS software and one-way Anova test.

Results: Hydatid cyst fluid, and BCG significantly inhibited the growth of tumors in comparison with control groups. Life spans of the mice in case groups were also estimated. The results revealed that in hydatid cyst fluid, and BCG injected mice, life span was significantly longer than that of control groups.

Conclusion: Hydatid cyst fluid has anti-melanoma activities and this effect may be related to immune response to parasite antigens.

Keywords: Melanoma, *Echinococcus granulosus*; Hydatid fluid, BCG.

Hard tick's diversity on sheep in Sabzevar city, Iran

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objectives: Some of ticks are major vectors of pathogens for animals and humans. This study was conducted to determine tick infestation rate of sheep and seasonal variations of tick population in Sabzevar city.

Materials and Methods: survey was carried out during April 2016 until March 2017. A total of 800 sheep were randomly selected and examined. A total number of 400 adult ticks were collected and then were preserved in 70% Ethanol alcohol with 5% glycerin and were identified using diagnostic keys.

Results: Based on the results obtained in this study, two Genera and four species including *Hyalomma anatolicum* (40%), *Rhipicephalus turanicus* (30%), *Hyalomma excavatum* (16.25%) and *Rhipicephalus bursa* (13.75%) were identified. The most prevalent tick was *Hyalomma anatolicum*. The prevalence of ticks on sheep was 22.5%. The highest amount of infestation was observed in spring (53%). In this study, the highest infestation rate was reported in the head and ear area with 49.5%.

Conclusion: Ticks are important due to their role in transmission of diseases such as theileriosis, babesiosis, anaplasmosis and ehrlichiosis. So the result of this study can be useful for implementation of disease control.

A study on prevalence of some helminthic infections of the liver among cattle and sheep in abattoir of Sabzevar, Iran

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Introduction and objectives: One of the most common parasites among humans and livestock is *Hydatid* cyst, *Dicrocoelium* and *Fasciola*. The aim of this study was to determine the prevalence of these parasites in cattle and sheep in slaughterhouse of Sabzevar, Iran.

Materials and Methods: This study was conducted to evaluate the presence of liver fluke in Sabzevar slaughterhouse during April 2014 till March 2016. In this study, referring to Sabzevar slaughterhouse, 1653 liver of cattle and 26720 liver of sheep were investigated for the presence of hydatid cysts and liver flukes.

Results: The results showed that (5.86%), (0.84%) and (5.68%) were infected with Hydatid cysts, *Fasciola* and *Dicrocoelium*, in cattle respectively and also among sheep's liver, (0.87%) (0.06%) and (2.13%) were infected with Hydatid cysts, *Fasciola* and *Dicrocoelium* respectively. The rate of infection with *Dicrocoelium* was higher in both livestock than in other parasites.

Conclusion: The results of this study indicate that the prevalence of the mentioned parasites is high, which causes high economic losses. Therefore, sanitary and preventive measures are essential.

Keywords: Zoonosis, Hydatid, Liver flukes, Sabzevar

Parasitic infections in immunocompromised hosts

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Abstract

Introduction: The opportunistic parasites are one of the main causes of mortality in the immunocompromised hosts. Parasitic infections are among the most of all widespread human chronic infections worldwide. Immune system confronts the parasites by its own special humoral and cell mediated immunity criteria in order to resolve the created inconvenience, therefore congenital and acquired immunodeficiency disorders can increase the ratio of mortality in as such cases.

Methods: In this review study preparation steps databases including Pubmed, Google scholar, Sciencedirect, Elsevier, Medscape have been searched.

Results: Studies prove that immunodeficiency disorders can lead the patients to perilous injuries caused by parasitic infection.

Discussion and Conclusion: Patients with obtained or congenital immunodeficiency show a number of various immune defects such as, T-Cell lymphopenia especially serious decreased numbers of CD4 T helper cells in Human Immunodeficiency Virus (HIV) infections, Hematopoietic malignancies, hypogammaglobulinaemia, immunosuppressive therapies and absence of eosinophilia in patients receiving corticosteroids all can cause severe different parasitosis including infections caused by *Cryptosporidium parvum*- the most opportunistic intestinal protozoa-, *Isospora belli*, *Giardia lamblia*, *Ascaris lumbricoides*, *Strongyloides stercoralis*, *Trichuris trichiura*, *Entamoeba histolytica*, *Taenia* species, *Hymenolepis nana*, *Enterobius vermicularis* that could be much less effective in immunocompetent conditions in hosts therefore screening and monitoring parasitic infections in the patients with the immune deficiency presentations is recommended.

Current Epidemiological Status of Visceral Leishmaniasis in Kaleybar and Khoda-Afarin Districts, Northwestern Iran

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Objectives: Leishmaniasis are a group of diseases that caused by species of a genus of parasitic and intracellular protozoan called *Leishmania*. Visceral leishmaniasis (VL) or kala-azar is a fatal form of leishmaniasis if left untreated. Two main foci of VL in Iran located in the north-west and south parts of Iran. We designed a cross-sectional study to clarify the sero-prevalence status of VL in Kaleybar and Khoda-Afarin districts, north-west of Iran.

Methods: A total of 1581 human samples (1420 children, 161 adults), 101 domestic dogs samples specimens were collected during 2016-2017. Collected human and dogs' sera were tested using direct agglutination test (DAT). Also, a structured questionnaire was applied to evaluate the correlation between potential risk factors and related clinical signs/symptoms with human and dogs' seropositivity.

Results: Totally, 2.18% children samples were positive at titers $\geq 1:800$, among them 13 cases (41%) were above $\geq 1:3200$. Anti-*Leishmania infantum* antibodies at titer $\geq 1:320$ were found in 9.90% dogs' samples. Also, anti-*Leishmania infantum* antibodies were not detected in sera of adults. Statistical analysis revealed significant correlations between presence of anti-*Leishmania infantum* antibodies and fever ($p < 0.001$), anemia ($p = 0.001$) and weight loss ($p = 0.016$) in children. On the other hand significant correlation was revealed between the *Leishmania* infection and shelter ($p = 0.039$), cutaneous lesion ($p = 0.005$), lymphadenopathy ($p = 0.001$) and weight loss ($p < 0.001$) in the infected dogs.

Discussion: The results indicated that visceral *Leishmania* infection is prevalent in rural areas of Kaleybar and Khoda-Afar districts where located in East-Azerbaijan province and active detection and treatment of VL cases should not be neglected.

Keywords: Visceral leishmaniasis, Human, Domestic dog, Seroprevalence, Risk factor, Iran

Comparison of Three Common Molecular Markers for Detection of *Leishmania* DNA by PCR

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Abstract:

Objectives: Leishmaniasis is a vector born disease, that be caused by different species of an intracellular protozoan parasite genus called *Leishmania*. The disease has different forms and is an important public health problem in tropical and subtropical countries. There various methods to diagnosis the disease including microscopic examination, culture, serological tests, and molecular detection of the parasite genome.

Methodes: In this study we tried to compare sensitivity of three molecular markers including kDNA, Cyt b and ITS1 for detection of *Leishmania* sp. genome. For this purpose, at first Standard strain of *L. major* MHOM/IR/75/ER was cultivated in RPMI 1640 (Gibco, Germany) medium with 20% FBS (Gibco) at 23 °C. After that, different numbers of amastigotes were separated from culture medium and their DNA were extract using DNA-plus extraction kit (SinaClon, Iran). PCR were conducted on kDNA, Cyt b and ITS1 genes.

Results: The results revealed that kDNA-PCR can detect the lowest number of the parasite (5 promastigotes) and was followed by Cyt b-PCR (10 promastigotes), and ITS-PCR (50 promastigotes).

Discussion: In conclusion, kDNA-PCR is the most sensitive method for detection of *Leishmania* DNA among these 3 genes, so it is the best choice for screening of leishmaniasis.

Keywords: *Leishmania*, PCR, kDNA, Cyt b, ITS

Study of the frequency and incidence of scorpion envenomation in Aghajri County, Khuzestan Province, southwestern Iran

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Abstract

Objectives: Scorpion sting is a medical problem and a life-threatening hazard in many parts of the globe including Iran. Scorpions are widely distributed in Iran. Sixty-four species have been identified in Iran, with at least 18 of these recorded in Khuzestan Province. Data on scorpion envenomation is available for many parts of Iran, but not for Aghajri County.

Material and methods: This study was based on 553 cases of scorpion stings submitted to the medical and health centers of Aghajri County, Iran, over a period of 4 years. Epidemiological information of victims in Aghajri was obtained from a standard data sheet and analyzed by SPSS 16 software.

Results: Total cases were residing in urban areas. Stings were found throughout the year with the largest seasonal incidence during the summer (46.3%) and the lowest in the winter (4.9%). Most cases of scorpion stings occurred during August and July.

Discussion & Conclusion: Our results indicate that scorpionism is common in Aghajri County, particularly during the summer. The highest rate of stings were recorded in urban areas, therefore, training programs should be noticed for preventing scorpion sting in urban areas. The majority of educational programs should be focused on housewives, because they can transfer what they learn to their children and thus play a significant role in controlling scorpion sting cases among students.

Keywords: Scorpion Sting, Epidemiology, Incidence, Iran.

Study of bacterial and fungal infection in cat ears due to *Otodectes cynotis* mite

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Objectives: The *Otodectes cynotis* mite or ear mite is seen in the ear canal of dogs, cats and wild animals. The parasite is a Psoroptidae mite. The provocations induced by this mite may alter the bacterial and fungal flora of the ear, causing inflammation, pain, redness and itching of the ear. This study aimed at determining the prevalence of bacterial and fungal infections in cats' ears with *Otodectes cynotis* mite.

Materials and Methods: In this study, 50 cats were sampled using macroscopic (Otoscopy) and microscopic techniques for diagnosis and identification of *Otodectes cynotis* and culture media for diagnosis of fungal and bacterial pathogens from ear discharge.

Results: Out of 50 cats, *Otodectes cynotis* was removed from five cats (10%), and bacterial and fungal infections was observed in the right and left ears of four cats with *Otodectes cynotis*. The bacterial and fungal infections were observed in 28 right ears and 29 left ears of 45 remaining cats.

Conclusions: The presence of *Otodectes cynotis* mite on a cat's ears can lead to imbalance in the bacterial and fungal flora, and the infection in the cat's ears with secondary bacterial infections (negative gram and positive gram) and fungal infections with (saprophytic fungi) was identified.

Keywords: *Otodectes cynotis*, fungal infections, microbial infections

Ancient *Fasciola* Spp. Egg in the Fifth-Millennium BC Cemetery in the Zohreh Prehistoric Project

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Abstract

Aim & Background: The burial sites can gather so many valuable information about ancient parasites. The site of *Tol-e ChegaSofla* in the Zohreh Prehistoric Project contributes to a wider understanding about Paleoparasites and maybe the origin of some parasitic diseases.

Material and methods: 3 burial sites were found between 2015 until 2016 in cemeteries of *Tol-e ChegaSofla*. The samples taken from Pelvic region of the human skeletons and rehydrated in the Na₃PO₄, after 10 days samples were examined with the light microscope.

Results: In one slide we found ancient *Fasciola* Spp. egg.

Discussion&Conclusion: This is first recording of ancient eggs of *Fasciola* spp. in west of Asia. Although Humans are not preferred host for *Fasciola* Spp. but this finding can show us that, there were suitable conditions for developing host-parasite relationships and humans can be affected too. It seems the *Tol-e ChegaSofla* cemeteries can give us interesting information about prevalence of *Fasciola* Spp. in the south west of Iran. Because of amount of samples this study has been continued.

Keywords: Paleoparasites, Tol-e ChegaSofla, ZohrehPrehistoric Project, *Fasciola* Spp.

An Epidemiological Study on the Status of Hydatid Cyst in Surgical Patients in Golestan Hospital of Ahwaz during 2002-2011

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Abstract

Aim & Background: Hydatidosis is one of the most important zoonotic parasitic diseases. Hydatidosis is endemic in Iran and is the cause of hospitalization of almost 1% of patient's insurgical wards. The purpose of this study is to examine the epidemiologic status of hydatid cyst in patients undergoing surgery in Golestan hospital of Ahvaz during 2002-2011.

Material and methods: This research is a cross-sectional study. During the mentioned period, 2002 until 2011, 55 patients in Ahvaz Golestan hospital have undergone hydatid cyst surgery and the information in the patients' files was examined by referring to the relevant archives in the mentioned hospital.

Results: Among the 55 patients studied, 37 (67.3%) were female and 18 patients (32.7%) were male. The highest incidence rate was found in liver with 47 cases (85.5%), followed by lung with 5 cases (9%). Considering the results, the highest prevalence rate was found among urban residents (n=33, 60%) whilst 22 cases (40%) belonged to the rural residents.

Discussion & Conclusion: It seems the residences of urban areas because of their access to medical centers, has more chance to diagnosis their hydatidosis. The results of this study indicate that the occurrence of the disease was significant in Khuzestan province during the mentioned period which reflects the necessity of more comprehensive and updated studies.

Keywords: Hydatidosis, Hydatid cyst surgery, Ahwaz Golestan Hospital, Epidemiology

Memory Impairment and latent *Toxoplasma gondii* Infection; Association, Awareness and Risk Factors in a High *Toxoplasma*-Seroprevalence Community

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Abstract

Introduction: Memory impairment (MI), happens when a person loses the ability to remember information and events they would normally be able to recall. Toxoplasmosis is one of the most important parasitic diseases involving the brain. The present study was conducted to investigate the probable association between MI and toxoplasmosis using serological and molecular techniques.

Materials and methods: The study population consisted of 87 Alzheimer's patients (AP) and 87 healthy controls which were selected under the supervision of neurology consultant. The *Toxoplasma*-specific antibodies were measured using commercial ELISA kits. The desired region for *Toxoplasma* B1 gene was amplified by using specific primers and a thermocycler. Specificity of primers was confirmed by direct sequencing, aligning and phylogenetic analysis of the amplicons.

Results: Prevalence of toxoplasmosis in AP and control group was 66.6% and 56.3% ($P=0.99$) and 52.8% and 40.2% ($P=0.229$) using ELISA and PCR respectively. Despite the higher prevalence of toxoplasmosis in AP compared with the controls, a significant relationship was not found between MI and toxoplasmosis. The multiple sequence alignment of *T. gondii* isolates revealed a common haplotype.

Discussion and conclusion: The significant relationship between some variables and toxoplasmosis as well as the MI could reveal the risk factors for MI. These results provide fresh insights into the ambiguous association between *T. gondii* infection and MI. As a probable or concomitant risk factor, toxoplasmosis could induce the MI, principally in patients with the chronic or latent infection.

Keywords: Memory impairment, Alzheimer's disease, probable link, seromolecular, toxoplasmosis.

Seroepidemiological study of Toxoplasmosis in women in Dorood city of Lorestan province in 2015

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Abstract

Introduction: Toxoplasmosis is a disease that is caused by infection with an intracellular protozoan parasite called *Toxoplasma gondii*. The embryo of pregnant women is more at risk and sometimes has irreparable complications in the fetus. This study was conducted to determine the seroepidemiology of this disease in women of Dorood city in 2015.

Materials and Methods: In this cross-sectional study, all women in Dorood city were enrolled in the study with prior consent. The sampling method was a multi-stage and consolidated cluster sampling method. After completing the information forms containing demographic variables, the blood samples were collected from the women. ELISA was used to find serum antibodies against *Toxoplasma*. Then, the results were analyzed statistically.

Results: Of 233 subjects who were included in the study, 72 (30.2%) had a positive serological test for toxoplasmosis. The seropositivity rate in the age group above 40 years (41.1%) was the highest and in the age group lower 20 years (8.5%) was the lowest, which was statistically significant ($p < 0.001$). The seropositivity rate was highest in employed people (75%) and in students with the lowest (5.9%), which was statistically significant ($p < 0.001$). There was no significant difference between the result of serological test and other variables in the subjects.

Discussion and conclusion: Due to the relatively high prevalence of toxoplasmosis in women in Dorood, the probability of occurrence of congenital toxoplasmosis and its complications in the fetus is expected. In these people, precautionary measures should be taken more seriously to reduce the risk of congenital toxoplasmosis.

Keywords: Seroepidemiology, Toxoplasmosis, Women, Dorood city, Lorestan province

A Review of Some Studies Done on the Epidemiology and Outbreak of Malaria in Different Regions of Iran (1382-1397)

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Background and aim: Malaria is one of the major health problems in tropical and sub-tropical countries, including Iran. The purpose of this article is to review some of the studies done on the epidemiology and outbreak of malaria in different parts of the country, and at different times for some provinces.

Material and Methods: From the scientific databases such as the SID and Magiran, 30 published articles during the years 1382 to 1397 were collected and the epidemiology of malaria in some provinces was investigated for the following cases: The method of screening, the method of study, the used software and statistical methods, the number of patients with malaria, the percentage of male and female patients, the percentage of Iranian and non-Iranian people, the status of epidemiology, the type and percentage of plasmodium parasite and finally the trend of eradication of the disease.

Results: Malaria is known as the most common transmitted disease by mosquitoes in the south and south-east of Iran, especially the provinces of Sistan and Baluchestan, Hormozgan and Kerman.

Anopheles stephensi, *Anopheles delta*, *Anopheles superpictus*, *Anopheles fluviatilis*, *Anopheles coliophasis* are the vectors of malaria in Iran. More than 90% of the plasmodium parasites are the type vivax and the rest of the *falciparum* and mixed species. Most malaria cases are males and aged between 20 and 30, and the outbreak of disease is more than the type of imported transmission. According to the World Health Organization's 2017 survey, there were 57 indigenous malaria cases and imported malaria cases in Iran, indicating a significant reduction in the number of malaria patients in Iran.

Discussion and conclusion: Climate conditions, rivers and water resources, stagnant waters, fountains and rivulet, palms, existence of agricultural fields, temperature, precipitation, relative humidity, intensity and direction of wind are the most important climatic factors affecting growth, propagation and the completion of the *Plasmodium* parasite and the anopheles mosquitoes are effective in the onset and outbreak of malaria. Immigration, pilgrimage, tourism attractions and job opportunities and ultimately traveling from other provinces and neighboring countries are effective in the transmission and spread of malaria. According to the WHO, with operational programs, Iran is one of the eastern Mediterranean countries, which indigenous malaria will be completely eradicated by 2020.

Keywords: Malaria, Epidemiology, *Anopheles*, *Plasmodium falciparum*, Vivax, Climate condition, Iran.

Prevalence and Genetic Characterization of *Cryptosporidium* in Children with Diarrhea

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Abstract

Background: Cryptosporidiasis is a worldwide zoonotic parasitic disease found in children and HIV-positive individuals and is mainly accompanied with diarrhea. This study attempted to compare the sensitivity and specificity of acid-fast staining, PCR, and ELISA methods for determining *Cryptosporidium* and its predominant species in diarrheal stool samples of the children.

Methods: In total, 221 diarrheal stool samples were collected from children who referred to Motahari Hospital in Urmia City, North-West of Iran. Acid-fast staining and ELISA were used to analyze all the samples, while the PCR method was considered as the golden standard of this study. Positive samples shown by the PCR were sequenced to determine the *Cryptosporidium* species. The three methods were compared regarding statistical factors, sensitivity, specificity, positive and negative predictive values, time duration, and experiment costs.

Results: According to our results, of 221 analyzed samples, four and seven samples were positive for *Cryptosporidium*, as indicated by acid-fast staining and PCR methods, respectively. The sensitivity and specificity of acid-fast method showed to be 57.14% and 99.53%, respectively, and five out of 94 samples were diagnosed as positive by ELISA (with 71.4% sensitivity and 100% specificity).

Conclusion: Our findings showed that the ELISA has a higher sensitivity and positive predictive value than acid-fast staining method for detecting *Cryptosporidium* parasite. Although having higher sensitivity and specificity in comparison with ELISA method, PCR needs more time and cost; therefore, ELISA is preferred for laboratory routine works.

Keywords: Acid-fast, *Cryptosporidium*, ELISA, PCR, Sensitivity, Specificity

Prevalence and Genetic Characterization of *Cryptosporidium* in Pre-Weaned Cattle in Urmia (Northwest Iran)

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Abstract:

Introduction: Cryptosporidiosis is a zoonotic disease that causes digestive problems in pre-weaned calves. Considering the zoonosis and veterinary aspect we evaluated the prevalence and genotyping of *Cryptosporidium* spp. in diarrheic pre-weaned calves in the northwest of Iran.

Material and Methods: A total of 100 stool samples of the infant calves with diarrhea were collected from industrial and conventional livestock farms in Urmia City. All the samples were tested with acid-fast staining, ELISA, and PCR. Positive samples of the PCR method were sequenced to determine the *Cryptosporidium* species. The obtained results were compared for the mentioned methods based on statistical factors, sensitivity, specificity, positive and negative predictive values, as well as duration of the experiment and the costs of testing.

Results and Discussion: The results of this study showed that the prevalence of *Cryptosporidium* spp. in diarrheic infant calves in Urmia City was 5%, and *C. parvum* species of *Cryptosporidium* was detected in all the sequenced samples. Based on the findings of the current study, the most appropriate method for the detection of the parasite is the ELISA that has a higher sensitivity and predictive value than acid-fast staining method and should be used in veterinary laboratories.

Keywords: Acid-fast, *Cryptosporidium*, ELISA, PCR, Sensitivity, Specificity

Morphologic and molecular study on nematodes infection in abomasum of sheep

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Introduction: The nematodes infections in sheep have important impact on economic and public health. This study was undertaken to evaluate morphologic and molecular nematodes infection in sheep. The first human infection report with *Haemonchus contortus*, *Marshallagia marshalli* and *Ostertagia ostertagi* was from Isfahan. In Iran, a few molecular studies reported on *Haemonchus spp.*, *Parabronema skrjabini* and *Ostertagia spp.*

Methods: A total of 240 abomasa of slaughtered sheep were randomly collected. The nematodes were morphologically identified. The genomic DNA of nematodes was extracted and 300-bp-fragment of ITS2-rRNA gene was amplified by PCR.

Results: Overall infection rate was 66.7%. Morphologically, infection rate for *H. contortus*, *M. marshalli*, *M. occidentalis*, *O. circumcincta* and *P. skrjabini* was 0.04%, 43.7%, 2.5%, 15.5% and 5%, respectively. Molecular characterization uncovered *M. marshalli* and *M. occidentalis* were same. *Haemonchus contortus* and *O. circumcincta* were from different species. The nucleotide sequences of *P. skrjabini* were similar to *O. occidentalis* and *M. marshalli*.

Discussion & Conclusion: Molecular findings on *M. marshalli* and *M. occidentalis* were in accordance with Dallas et al (2001). The sequences of *H. contortus* were different in 2-3 nucleotides. This was in agreement with Mashghi et al. (2015). The similarity of *O. circumcincta*, *O. occidentalis* and *O. trifurcata* was 97%-98% with difference of 2%-3%. Morphologic and molecular findings were different for *P. skrjabini*. Hasheminasab et al. (2016) reported the homology of various isolates of *P. skrjabini* was 68%-77%. Upon high similarity of nematodes' morphology, genotype characterization may be a reliable and informative technique to study in taxonomy.

Keywords: Nematode, Abomasum, Sheep.

Pathologic changes of abomasum due to naturally infected sheep with *Ostertagia circumcincta*

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Introduction: The parasitic infections of abomasa due to nematodes are importance of economic and public health worldwide and Iran. The present study was aimed to study histopathologic changes in abomasa of naturally infected sheep with *Ostertagia (O.) circumcincta*.

Methods: The naturally infected abomasa of sheep with *O. circumcincta* were collected for gross and microscopic observation. The tissue samples were fixed in 10% buffered formalin and stained with hematoxylin and eosin (H&E).

Results: Histopathologically, there was hyperplasia of mucosa, edema of the lamina propria with loss of parietal cells and replacement of mucous secreting cells. The severe infiltration of inflammatory cells, particularly eosinophil in connective tissue around abomasal glands as well as a few adult helminths was also observed. Chronic hypertrophic gastritis with presence of hypobiotic larvae was evident.

Discussion and conclusion: Histopathologic changes in abomasum of naturally infected sheep due to *O. circumcincta* was in accord with Scott *et al* (2000). Blanchard *et al* (1986) noted that there was glandular edema, focal infiltration of lymphocytes, hyperplasia of mucosa and thickness of abomasum. Public awareness programs are recommended to encourage sheep owners for using accurate anthelmintic medicines with correct repeats.

Keywords: Abomasum, *Ostertagia circumcincta*, Sheep

The Molecular Evidence of *Babesia lengau* Infection in Mice and Associated Ticks (*Acari: Ixodidae*) at Two Cheetah Breeding Centers in South Africa

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Abstract

Mice (Muridae), the largest family of mammals, occur worldwide. They host a variety of immature stages of Ixodid tick and *Babesia* species in South Africa. This study was conducted to determine the tick burdens and detecting possible *Babesia lengau* in mice and associated ticks at the Ann van Dyk Cheetah Breeding Centre and the Hoedspruit Endangered Species Centre.

This study was conducted between 2010 and 2011, during which 452 immature ticks were collected and 22 mice were trapped. Genomic DNA was extracted from collected ticks and blood samples from trapped mice and amplified the molecular biology laboratory in the Department of Veterinary Tropical Diseases, at the Faculty of Veterinary Science, The University of Pretoria.

The primers successfully amplified the V4 hypervariable region of the small subunit of the 18S rRNA gene of *Babesia* parasites. The combination of PCR and Reverse Line Blot Hybridization assay revealed that 7 mice and 308 ticks were infected with *Babesia lengau*.

The maintenance of *Babesia* species is dependent on two classes of hosts, the one a mammal and the other a specific tick vector which must feed on the mammal host. The results presented in this study have demonstrated the biological survival of *Babesia lengau* by its presence in the trapped mice and *Haemaphysalis elliptica* ticks at cheetah breeding centres and the risk of cheetahs to contract babesiosis. Also *Aethomys* species, compared to the other mice species, harbour the protozoan parasite *Babesia lengau*.

Keywords: *Babesia*, cheetah, murid rodents, South Africa, Ixodid ticks

***Dirofilaria immitis* Infection in Stray Dogs of Kermanshah by Immunochromatography**

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Background: Cardiopulmonary dirofilariasis is a cosmopolitan disease caused by *Dirofilaria immitis*, which affects mainly canids and felids. Moreover, it causes zoonotic infections, producing pulmonary dirofilariasis in humans. Heartworm disease is a vector-borne transmitted disease, thus transmission depends on the presence of competent mosquito species, which is directly related to favorable climate conditions for its development. Cardiopulmonary dirofilariasis is mainly located in countries with temperate and tropical climates. This study was conducted to evaluate the contamination in stray dogs of Kermanshah in 2015.

Materials and methods: Blood samples and serums were taken and prepared to be evaluated through immune chromatography with CHW Ag Test card kit.

Results: The result shows the frequency 11% by immune chromatography in stray dogs. The frequency of infection in the age of 6 years and upper and in male was higher but this difference was not significant statistically.

Conclusions: According to the result of this study and hygienic importance of *Dirofilaria immitis*, it is suggested to kill contaminated dogs, attention of livestock clinicians toward importance of the disease to simultaneous Knott and serologic test in referred dogs.

Keywords: *Dirofilaria immitis*; Kermanshah; Immune Chromatography

Lice and Mites Infestation in Bee-Eaters (Aves: Meropidae) from Western Iran

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Introduction: Three species of bee-eaters, i.e. Green Bee-eater (*Merops orientalis*), Persian Bee-eater (*M. persicus*) and European Bee-eater (*M. apiaster*) exist in Iran. Although bee-eaters are known as effective ecosystem engineers but they are facing different threats like hunting or poisoning by beekeepers in different parts of Iran. There is little information about ectoparasites of wild birds in Iran.

Methods: During April 2018, a total number of 45 dead bee-eaters (*M. persicus* and *M. apiaster*) (probably poisoned) were collected in farms located on Ilam city suburbs and were examined for ectoparasites and endoparasites. The removed parasites were preserved in ethanol 70%. The collected chewing lice and mites were mounted on slides for identification and further study.

Results: The only ectoparasites found on the examined birds were chewing lice and mites. A total number of 861 chewing lice (prevalence 100%) and 98 adult mites (prevalence 51.1%) were collected from 45 infested birds. Four species of three genera of chewing lice, i.e. *Brueelia apiastri*, *B. erythropteri*, *Meromenopon meropis*, *Meropocetus meropis* and two mites' species of *Piciformobia* spp. were identified.

Discussion and Conclusion: There are no previous studies on ectoparasites of Bee-eater birds in Iran, so this is the first report of lice and mites on *M. persicus* and *M. apiaster* in Iran. Although none of the ectoparasites found are serious threats to the bee-eaters health, the conflict between bee-eaters and beekeepers may lead to the population decline in these important birds. This may need public awareness and science communication with beekeepers.

Keywords: Ectoparasites, Bee-eaters, Mallophaga, Acari, Ilam

Experimental Visceral Leishmaniasis Induces Metabolic Imbalance in Mice Testicular Tissue: Evidence for Role of Monocarboxylate Transporter 4

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Protozoan parasitic diseases as endemic diseases can lead to infertility due to reproductive dysfunction. The aim of this study was to trace testicular monocarboxylate transporter 4 (MCT 4) following experimental visceral leishmaniasis (EVL) induction in BALB/c mice. In this experimental study, 18 adult male BALB/c mice were randomly divided into three equal groups including untreated control, sham (0.20 mL medium, intraperitoneally) and EVL groups. The EVL was induced through 0.20 mL intraperitoneal injection of 12×10^6 *Leishmaniainfantum* stationary-phase promastigotes containing solution. All mice in experimental groups were euthanized following anesthesia after 6 weeks and spleen and testes were removed immediately in a sterile condition and used for infection confirmation and immunohistochemical studies, respectively. The EVL resulted in a significant decline in MCT 4 production in BALB/c micetesticular tissue compared to control and sham groups. This finding indicated that EVL in BALB/c mice may lead to metabolic imbalance in testicular tissue through MCT 4 down-regulation.

Keywords: Monocarboxylate Transporter, Mouse, Testis, Visceral leishmaniasis

Scientometric Analysis of Research Activity and Collaboration in the Field of Parasitology from 2010-2019

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Background and objectives: Scientometric deals with measuring and analyzing scientific literature which is a sub category of bibliometric. The present study performed a scinometric evaluation in the field of parasitology.

Method: Using bibliometric analysis, we investigated all the scientific papers in the field of parasitology that were indexed in Web of Science (WOS) from 2010 to May 2019. This study aimed to examine the patterns of research in the field of parasitology. We have identified the main keywords, journals, authors and countries. Following the cluster analysis, the VOSviewer software (version 1.6.9) was utilized to draw the bibliometric maps.

Results: A total of 2845 publications about parasitology were retrieved from WOS and transformed to VOSviewer software after utilizing some data mining techniques. The most common document types were 'journal articles' with 82.81% (n=2356) of the total followed by 'reviews' with 10.86% (n=309). USA and Australia almost dominated the publications in this area each with 721 (25.34%) and 332 (11.67%) publications. The most prolific authors were GASSER RB and POULIN R with 21 articles each followed by MARTINS ML with 15 articles. Among 2845 publications, University of Melbourne (n= 73) and Centre National de la Recherche Scientifique Cnrs (n= 67) were the most active organizations. International Journal for parasitology with 1049 articles was the most prominent publisher in this area followed by International Journal for Parasitology drugs and drug resistance (n=170). The interdisciplinary analysis showed that parasitology (n=1857) is highly in relation with Veterinary Sciences (n=247) and environmental sciences ecology (n=216). However, among them Iran had 75 articles, mainly in the form of 'journal articles' (n=75), with major collaborations from Tehran University of Medical Sciences (n=19) and Islamic Azad University (13). The most prolific authors in Iran were BERENJI F and DARYANI A with 5 articles. It should be mentioned that Iranian Journal of Parasitology (n=27) and Jundishapur Journal of Microbiology (n=5) were the most active publishers.

Discussion and Conclusion: The findings of the current study shed light on the growth, collaboration and the spread of knowledge in the field parasitology. On the whole, our study highlighted the most frequent topic clusters and topics, the prominent authors and institutions and countries. Our findings suggest may encourage authors to submit more publications in the journals indexed in WOS and to index journals in the internationally recognized databases such as WOS to get high visibility.

Molecular identification of *Vermamoeba* and *Acanthamoeba* from Stagnant Water in Kashan, Central Iran

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Abstract

Introduction: Free-living amoebae (FLA) are the most abundant and widely distributed protozoa in the environment, including many genera *Acanthamoeba*, *Vermamoeba* and *Naeglaria*. These Amoebas can cause Granulomatous Amoebic Encephalitis (GAE) in immunocompromised patient. This study was conducted to isolate and identifies *Vermamoeba* and *Acanthamoeba* from stagnant water in Kashan.

Material and Methods: In this Cross-Sectional study a total of thirty- eight samples from Kashan's stagnant water were collected during 2015-2016 The samples were filtered in 0.45µm nitrocellulose paper and cultured on to 1.5% NNA for the presence of free living amoeba (FLA). After DNA extraction, Polymerase Chain Reaction was performed for detection *Vermamoeba* 18srRNA gene. Observation a 750 bp fragment was confirmed as *Vermamoeba*. *Acanthamoeba* spp. was identified by PCR using DJP1 and DJP2 primers and observation a 500 bp band in gel electrophoreses. The data were recorded in SPSS.16 and analyzed by X2 and Fischer Exact test.

Results: Out of 38 stagnant water samples, 18(47.4%) were positive for *Vermamoeba* and 20(52.6%) negative. Also, 27 out of 38 samples (71.1%) were confirmed as *Acanthamoeba*.

Conclusion: The pollution of stagnant water to *Acanthamoeba* was higher than *Vermamoeba*, then, increasing human awareness about disease transmission, health education and interventions to reduce and prevent of disease are recommended.

Keywords: *Vermamoeba*, *Acanthamoeba*, Stagnant water

In Silicoanalysis of a New Multiepitope Construct of *Leishmaniainfantum*

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Background and aims: With significant advances in bioinformatics in recent years, pre-laboratory studies can be very valuable and useful which will greatly predict the outcome of the trials. Therefore in order to improve the diagnostic status of visceral leishmaniasis, we decided to design a chimeric component composed of eight high antigenic *leishmaniainfantum* epitopes and investigate its efficiency. Thus, it should also be studied in a laboratory if the result is desirable.

Materials and Methods: Important immunogenic antigens (GP63, HSP-70, CPA, H2A, H3, KMP-11, TRYP and LACK) in *leishmaniainfantum* parasite were searched for their established epitopes in IEDB Immunodata web server. The best linear B cell epitope was gained for the six antigens but for the other two, prediction was done. After designing the multi-epitope structure, its two and three-dimensional structure was determined by use of bioinformatics tools. Also, its interaction with human IgG was examined by HADDOCK.

Results: In silico analysis showed that the multi-epitope construct has an appropriate two- and three-dimensional structure and has the ability to create a specific binding to human immunoglobulin.

Conclusion: According to the computer base analysis it could be concluded that the new chimeric protein construct has potential to study in vitro. Anyway this experience has been productive in laboratory too because the same results were achieved.

Keywords: Linear B cell epitopes, *Leishmaniainfantum*, prediction, Computational software

Golden Jackal (*Canisaureus*) as Indicator Animal of *Trichinella britovi* in Iran

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Abstract

Introduction: Nematodes of the genus *Trichinella* are zoonotic parasites causing trichinellosis. In Iran, these parasites occur in several animal species and rare human cases have been recorded.

Methods: To monitor the epidemiological pattern of these parasites in the Khorasan- e- Razavi province, Northeastern Iran, muscle tissues were collected from the tongue of 295 stray dogs, one red fox (*Vulpes vulpes*), 12 golden jackals (*Canisaureus*), and one wild boar (*Susscrofa*) roadkill in 2016-2017. *Trichinella* spp. larvae were retrieved using the artificial digestion method and identified at the species level by multiplex PCR.

Results: Larvae identified as *Trichinella britovi* were detected in five stray dogs (1.7%) and one golden jackal (8.3%). The results confirm the circulation of *T. britovi* in animals of the Khorasan- e- Razavi province as previously documented.

Conclusions: A review of the literature on *Trichinella* spp. in animals of Iran showed that these parasites were previously detected in 20.02% and 0.04% of carnivore and omnivore mammals, respectively, and that golden jackals can be screened as indicator animal of these zoonotic nematodes. Convenient sampling of *Trichinella* susceptible roadkill animals may provide a good method to monitor the circulation of these parasites within any given region.

Keywords: *Trichinella britovi*; Carnivore; Iran; Multiplex PCR ; epidemiology

Species Identification of Cutaneous Leishmaniasis in Quchan, Northeast of Iran

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Abstract

Introduction: Khorasan province is an endemic focus for cutaneous leishmaniasis. Species identification of *Leishmania* protozoa is useful for the control and prevention of leishmaniasis. This is the first study of the identification of *Leishmania* species with the evaluation of some risk factors in Quchan city, Northern Khorasan, Northeast of Iran.

Methods: Questionnaire and slide smears were obtained from 103 individuals who suspected to the leishmaniasis. Optimized PCR method was performed using specific kDNA primers on all slides. Data were analyzed with SPSS ver.20 software.

Results: Among 103 subjects with skin ulcers suspected to CL, 77 (74.8%) were positive in direct microscopic smear. However, specific *Leishmania* PCR bands were observed in 86 (83.4%), which 57 subjects had *L. tropica* and 29 has *L. major*. The most age range involved was 20-30 years and the most common site of lesions was the hands. From 57 cases of *L. tropica* 43.9% and 56.1% were in urban and rural district, respectively. Sensitivity of microscopy method for diagnosis of *Leishmania* spp. was calculated 89.5% in this study.

Conclusions: *L. tropica* is the dominant causative species for cutaneous leishmaniasis in Quchan city. It has identified a new rural focus of cutaneous leishmaniasis caused by *L. tropica* in Quchan suburb.

Keywords: *Leishmania*, PCR, Quchan, Species, Iran

Frequency of Toxocariasis among Asthmatic Children in Northeastern Iran

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Abstract

Introductions: Toxocariasis is a zoonotic and telluric disease caused by *Toxocara* species mostly in tropical areas. The relationship between toxocariasis and asthma has always been a subject for discussion. This study evaluated the seroepidemiology of *Toxocara* among asthmatic children.

Methods: This cross-sectional study evaluated 150 children aged between 3-12 years with asthma presentations, who were referred to Dr. Sheikh Hospital of Mashhad University of Medical Sciences since April 2017 to March 2018. Serum samples were tested for the presence of anti-Toxocara antibodies using enzyme-linked immunosorbent assay (ELISA), and positive serums were confirmed by Western blotting (WB) method.

Results: Out of 150 asthmatic patients, 2 (1.3%) and 1 (0.6%) exhibited Toxocara immunoglobulin G (IgG) antibodies responses by ELISA and both methods, respectively. Moreover, none of the patients were detected as hypereosinophilia. **Conclusions:** It seems there are no significant relationship between Toxocara infection and asthma in Northeastern Iran. These findings suggest the need to perform Western blotting immunodiagnosis as well as ELISA, using Toxocara antigens to improve human toxocariasis diagnosis in patients with asthma.

Keywords: Asthma, Children, ELISA, Toxocara, Western blotting, Antibody

Frequency of *Acanthamoeba* in Water Sources of Bandar Abbas, Iran

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Abstract

Background: Free-living amebas are widely distributed in soil and water, particularly members of the genera *Acanthamoeba*. They can be pathogen to CNS, causing granulomatous amebic encephalitis (GAE) and keratitis.

Objective: There are limited information about these organisms in Hormozgan province especially capital city of Bandar abbas. This study aimed to investigate the free living amoeba in water sources and the research is still underway.

Material and method: In this descriptive cross-sectional study, 35 samples of tap-water of hospitals and dormitories accompanied by city fountains collected in Bandar abbas Hormozgan province, Iran. Free residual chlorine, pH, and temperature of samples were evaluated by kit and thermometer. After filtration by membrane of 0.45µm, samples were cultured on non-nutrient agar associated with bacteria. Then we sought for existence of *Acanthamoeba* by invert microscope.

Results: Our study and analyses showed that based on morphological characteristics of trophozoite and cysts, in total 14% of samples are positive for *Acanthamoeba* parasite.

Conclusion: Study showed that the frequency of parasite is slightly high and considerable. The data obtained from the study may be beneficial for the clinicians and the environmental professionals.

Keywords: *Acanthamoeba*, water sources.

Morphological Characterization of *Fasciola Spp.* Isolated from Different Host Species in Markazi Province, Central Iran

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Background: Fascioliasis is an important parasitic disease which is common between human and animals and has global distribution. It is very important in point of medical, veterinary and economy. Fascioliasis is caused by genus of Fasciolidae (*Fasciola hepatica* and *F. gigantica*). Iran is an endemic area and infection of this trematode is reported from all over all the country. The current study aimed to detect morphological and morphometrical characteristics of *Fasciola spp.* collected from different hosts in the abattoir of Arak, Iran during 2017.

Materials and Methods: In total, we examined 120 fresh adult flukes were collected from livers of infected goats, sheep, and cattle through necropsic analysis at the slaughterhouse. We identified *Fasciola spp.* based on morphological and metric assessment of external features as well as internal anatomy of worms using trematodes systematic keys. Statistical analysis was conducted using the one-way ANOVA and Student's t-test implemented in SPSS 18.0, and the significance level was <0.05 .

Results: The differences between the body length, area of the body, peripheral of the body, sucker area, cone length, cone width, in two species were significant ($P < 0.05$). Based on morphological characterization and morphometric parameters specially BL/BW ratios the flukes were grouped into *F. hepatica*, *F. gigantica*. Based on morphological characterizations, 111 (92.5%) and 9 (7.5%) specimens had the morphological features of *F. hepatica* and *F. gigantica*, respectively. The mean length and width as well as BL/BW ratios of *F. gigantica* was greater than *F. hepatica* in three examined hosts.

Conclusion: For detection *Fasciola* species in endemic area using morphology methods is necessary but merely, is not efficient for determination of genotyping.

Keywords: *Fasciola hepatica*, *Fasciola gigantica*, Morphological Characterization, genotype, Slaughtered Animal

Prevalence of the *Trichostrongylus* Species from Slaughtered Sheep and Goats in Kashan, Central Iran

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Introduction: The most important parasitic nematodes of ruminant's digestive tract belong to the family of trichostrongylidae with a worldwide distribution. There are more than 30 species of *Trichostrongylus* most of which are parasites of herbivores. The prevalence of *trichostrongylus* in sheep and goats improves control measures and reduces the mortality of the population. To identify species of parasites and also due to lack of sufficient information in this area, this cross sectional survey was carried out to determine the prevalence infection of the *Trichostrongylus* species in slaughtered sheep and goats at Kashan, abattoir located in central, Iran.

Materials and Methods: In total, we evaluated 154 (154.2%) gut of sheep and 130 (45.8%) gut of goats through necropsic analysis at the slaughterhouse during 2018. For determination species of *Trichostrongylus*, a total of 70 the parasite was collected and preserved in %70 alcohol and then observed under the microscope. For the measurement of worms, the microscope was equipped with a calibrated ocular lens. Then, worms, using nematodes systematic keys were identified. Analysis of variance test was applied for the statistical analysis, and the significance level was <0.05.

Results: A total of 26 (9.15%) out of 284 slaughtered animals were found were found positive for at least one of the *Trichostrongylus* (C. I= 9.15±3.35). The prevalence infection in sheep and goats were 6.5% and 12.3%, respectively (P=0.0181). Four different species of *trichostrongylus* including *T.colobriiformis*, *T.vitrinus*, *T.capricula*, and *T.probolorus* were isolated and identified. Percent prevalence for *T.colobriiformis*, *T.vitrinus*, *T.capricula*, and *T.probolorus* were 48.7% (sheep; 20.5%, goat; 28.2%), 25.7% (sheep; 10.3%, goat; 15.4%), 12.8% (sheep; 7.7%, goat; 5.1%) and 12.8% (sheep; 0%, goat; 12.8%) respectively.

Conclusion: The data in the current research work may provide a baseline for the future strategies of controlling of these parasites.

Keywords: *Trichostrongylus*; Morphology; Prevalence; Ruminant; Iran

The Effects of Chitosan-Titanium Dioxide-Glucantime Nanoassemblies on Amastigotes of *Leishmania Major*

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Introduction: *Leishmania* is a major global health problem. Pentavalent antimonial compounds such as meglumine antimoniate are still the first line treatment against leishmaniasis. These compounds have many disadvantages including side effects for vital organs, the long duration of treatment coarse and clinical resistance. The present study was to design nanoassemblies of chitosan-titanium dioxide nanoparticles loaded with glucantime for using their synergistic effects and enhancing the toxic effects of glucantime on *Leishmania* parasites.

Material and Methods: The nanoassemblies were prepared by electrostatic interactions and optimized by a response surface central composite design. The effects of glucantime, chitosan and TiO₂ NPs amounts were studied on the particle size, zeta potential, loading efficiency, and release efficiency of drug from nanoassemblies. The conjugation of nanoassemblies was verified by UV spectroscopy and changes in surface charge of NPs. The anti-amastigotes effect of glucantime loaded in TiO₂/chitosan nanoassemblies was studied by counting the average number of parasites per infected J774 macrophages in 100 cells.

Results: The optimized formulation obtained by using 12.5 mg glucantime, 25 mg chitosan and 6 mg TiO₂ NPs. Although TiO₂ NPs alone were effective more than negative control in reduction of amastigotes but they didn't show significant difference compared with free glucantime ($p>0.05$). However, at the concentration of 50 µg/ml and after 72 hours exposure nanoassemblies decreased the proliferation of *L. major* amastigotes 13 and 4-fold, compared with glucantime alone.

Conclusion: According to the details nanoassemblies have been synthesized based on differences of surface charge of materials with the nano particle size and zeta potential of them was modified. The in vitro antileishmanial effect of nanoassemblies indicated that they had much more effective than Glucantime and titanium dioxide alone.

Keywords: Glucantime; chitosan; titanium dioxide nanoparticles; promastigote; amastigote; *Leishmania major*

Parasitic Helminths as a Potential Therapeutic Candidate in Allergic Asthma

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Parasitic infections are a major theme in the “hygiene hypothesis”, as allergies and autoimmune diseases are less prevalent in countries with higher burdens of helminths and other parasitic organisms. Inverse relationship between helminths infection and immune-mediated disease has inspired researchers to use immuneomodulatory potential of helminths towards reducing symptoms of immune mediated diseases. These observations, as well as, experimental data originated from mouse model have led to introduce the helminths as therapeutic agent for inflammatory bowel disease (IBD), multiple sclerosis (MS), Rheumatoid arthritis (RA), diabetes and allergic asthma. Treatment of human immune-mediated diseases with live helminths appears to be valid and well characterized but it has several drawbacks such as accidental infection, reduced immune response to viral, bacterial and protozoal infection and increased susceptibility to them, reduced efficacy of vaccine, atopic reaction and poor patient acceptance with consuming eggs or live worms . One solution for this problem might be the use of Excretory/Secretory and somatic products of helminth. Although the mechanism of helminth therapy in inflammatory diseases is not clear, one possible mechanism may be the ability of helminth to produce soluble antigens that can modulate host immune response and suppress the innate and adaptive immune response to helminths and unrelated antigens. Here we summarize antiallergic/anti-inflammatory effects of helminths together along with our own study of the effects of *Marshallagia marshalli* on allergic asthma in a murine model. We also discuss possible mechanisms of helminth-induced suppression according to the recent advances of immunology.

Evaluation of in vitro Anti-Leishmaniasis Activity of Some Marine Species from Persian Gulf

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Introduction: Recently, the drugs with marine resources have been considered due to the lower costs and greater compatibility with patients. In the present study, the extracts of three marine organisms from the Persian Gulf; *Holothuria parva*, *Ophiocoma scolopendrina* and *Echinometra matheai*, were prepared and evaluated their in vitro anti-leishmaniasis effects.

Materials & Methods: All three marine organisms were collected from the coastal waters of Bushehr province and after separating their shell from the viscera, were extracted by Percolation method using different solvents. All prepared extracts were evaluated for cytotoxicity and anti-leishmanial activity on vero cells and promastigotes of *Leishmania major*, respectively using a biochemical MTT assay test.

Results: The results showed that by increasing the concentration of methanolic extracts in all three organisms, the cytotoxicity levels decrease for vero cells, as the lowest toxicity was observed at the highest concentration (800 µg/ml). Also, the highest anti-leishmanial activity was related to methanolic extracts of *Holothuria parva*'s shell (88.65%) and *Echinometra matheai*'s viscera (87.48%) at 800 µg/ml concentrations, and the lowest activity was obtained in aqueous extract of *Ophiocoma scolopendrina* (43.01%) at 20 µg/ml concentration.

Conclusion: Considering the anti-leishmanial effects of all three marine organisms studied in this research, and the presence of their rich reserves in the Persian Gulf, on the other hand, by performing a series of complementary studies in vivo and identifying the effective compounds of these extracts, efficient steps can be taken to produce effective drugs for the control and treatment of cutaneous leishmaniasis.

Keywords: Anti-leishmanial activity, *Holothuria parva*, *Echinometra matheai*, *Ophiocoma scolopendrina*.

Prevalence of Fasciolosis and Dicrocoeliosis from Slaughtered Animals in Arak, Iran

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Introduction: Trematode infections such as dicrocoeliosis and fasciolosis in farm animals are an important parasitic disease cause serious economic losses to livestock worldwide. *Fasciola* species and *Dicrocoelium dendriticum* are recognized as the most common liver flukes through many regions of Asia including Iran. We performed the current research to assess the prevalence rate of these infections among slaughtered animals from an economic perspective in Arak, Iran.

Materials and Methods: In total, we evaluated 369,790 slaughtered animals (118,463 sheep, 207,652 goats, and 43,675 cattle) in the abattoirs of Arak resulting from liver condemnation through postmortem analysis during 2016. *Fasciola* and *Dicrocoelium* parasites were evaluated in the removed liver and gallbladder. The species of parasites were identified, using the morphological characterization of *F. hepatica*, *F. gigantica*, and *D. dendriticum*. The prevalence of fasciolosis and dicrocoeliosis was determined. Analysis of variance test was applied for the statistical analysis, and the significance level was <0.05 .

Results: In total, *Fasciola hepatica* and *Dicrocoelium dendriticum* were detected in 0.56% (confidence interval, CI, 0.54-0.59) and 0.77% (CI, 0.75-0.81) of the animals, respectively ($p=0.1$). The prevalence of *Fasciola* infection was 0.75%, 0.42%, and 0.76% in sheep, goats, and cattle, while the prevalence of *Dicrocoelium* infection was 1.14%, 0.60%, and 0.60%, respectively. However, the difference in prevalence rates of fasciolosis ($p=0.21$) and dicrocoeliosis ($p=0.16$) was not significant in various hosts. The prevalence of *Fasciola* infection in all hosts was the highest during winter, and the difference was significant ($p=0.04$). However, no seasonal trend was found in the prevalence of dicrocoeliosis, and differences were not of significance ($p>0.1$).

Conclusion: Overall, fasciolosis and dicrocoeliosis in Iran always remain common in sheep, goats, and cattle that afford major economic loss of all the country also exist in Arak province. The present study could provide basic information for further examination of liver fluke infections in Iran.

Keywords: Dicrocoeliosis, Fasciolosis, Prevalence, Iran, Slaughtered animal

Antileishmanial Activity of *Perovskia abrotanoides* Terpenoid Fractions on Promastigotes of *Leishmania Major*

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Introduction: Leishmaniasis is an infectious disease developed by different species of protozoan parasites of the genus *Leishmania*. Pentavalent antimony is currently used for treatment of Leishmaniasis. Plant extracts provide a rich source of new drug agents in leishmaniasis endemic countries. *Berazambel* (*Perovskia abrotanoides*) is related to the family of Lamiaceae. In this research the killing effects of terpenoid fractions on *Leishmania* promastigotes were studied.

Material and Methods: The plant was collected around the city of Kashan, Isfahan province. Powder extract of aerial part of *Perovskia abrotanoides* was prepared by maceration method using water: ethanol solvent (80:20) and concentrated as much as possible and fractioned by MPLC method using silica gel adsorbent RP18 columns. From the obtained fractions terpenoids were selected and turned into dry powder to evaluate anti *Leishmania* effects of them.

Results: Data proved anti *Leishmania* effects of terpenoid fractions against *L. major* promastigotes was dependent on the fraction dose and time.

Discussion: It is necessary to evaluate the anti *Leishmania* effect of these fractions in animal model and volunteer humans.

Key word: *Leishmania major*, *Perovskia*, Terpenoid, Promastigotes

Prevalence Intestinal Infection of Parasites in Patients Referred to Laboratory in Tabriz Healthcenter Laboratory, Since March 2018 to May 2019, Tabriz, Iran

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Introduction: Intestinal parasitic infection is a major health problem in many developing countries. Parasites were the most important cause of debilitating disease as epidemic and endemic in all over the world especially among the children and adults in both urban and rural lifestyles.

Material and method: This study was done among different age group (4 to 65 years old) referred to laboratory in Tabriz health center laboratory. In this study, parasitologic methods such as the direct assay method and formalin-ether concentration method were used for diagnosis of worm eggs, cysts, and protozoa trophozoite. Susceptible and protozoan positive samples were stained using the Trichrome staining method.

Result: Eventually 97822 samples which sent to this laboratory since March 2018 to May 2019. Helminth and protozoan infection were observed in 51 (0.05%) and 587 (0.6%) participants, respectively. The helminthes infection was caused by *Enterobius vermicularis*, *Ascaris lumbricoides* and *Taenia saginata* in 48(0.05%), 2(0.002%) and 1 (0.001%) cases, respectively. Protozoan infection was also caused by *Entamoeba coli*, *Giardia lamblia*, *Blastocystis hominis*, *Chilomastix mesnili*, *Entamoeba histolytica/dispar*, *Endolimax nana*, *Iodamoeba buetschlii* and *Trichomonas hominis* in 167 (0.17%), 298 (0.30%), 61 (0.06%), 6 (0.006%), 11 (0.01%), 9 (0.009%), 11(0.01) and 24(0.02%) patients, respectively.

Conclusion: Prevalence and frequency of intestinal parasites among different age group demonstrated to emphasis transmission of these parasites. Helminthes infection has decreased drastically but protozoan infection is still considered a health problem in this study.

Key Word: Tabriz health center laboratory – Prevalence - Intestinal parasites

Determination of Visceral Leishmaniasis Infection in Asymptomatic Dogs by non-Invasive Sampling and PCR Targeting by ITS-rDNA Gene in North Khorasan Province

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Aims: Detection of visceral leishmaniasis and the variation among isolated *Leishmania* species in asymptomatic dogs in North Khorasan, Iran; using less invasive and sensitive diagnostic methods.

Methods: Sampling was performed from blood, snout and conjunctiva of asymptomatic sheep-dogs using non-invasive method in North Khorasan. DNA of canines was extracted and PCR was amplified in a region of the ribosomal RNA amplicon of *Leishmania* (ITS-rRNA gene), which was shown to be species-specific by DNA sequence.

Results: Total of 118 samples was obtained from 37 asymptomatic dogs which of them, 11 dogs were infected. None of the 11 dogs were simultaneously infected in all conducted samples (Blood, snout, right and left conjunctiva). From the 11 infected canines 21 samples were positive for *Leishmania* parasites. *Leishmaniainfantum* was the only *Leishmania* that was detected among the canines' population in North Khorasan province.

Conclusions: Visceral leishmaniasis usually observed in children under the age of 10 years. The highest number of infections was in the age group of 5-10 years old of dogs which probably states that this group is more sensitive to VL in this region. Although the bone marrow and lymph nodes have a lot of parasites, according to studies, conjunctiva, oral mucosa or snout and blood are both reliable and suitable for molecular studies. In addition, this method is non-invasive and ethical for the animal. In this survey, based on the ITS-rDNA gene and sequencing, *Leishmaniainfantum* was confirmed as the causative agent of visceral leishmaniasis in North Khorasan province.

Keywords: *Leishmania* parasite, asymptomatic dog, non-invasive, ITS-rDNA

Phylogeography and Genetic Diversity of Human Hydatidosis in Bordering the Caspian Sea, Northern Iran

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Background: Human echinococcosis acyclo-zoonotic infection caused by tapeworms of the *Echinococcus granulosus sensu stricto* complex. The detection of mitochondrial genome data of genus *Echinococcus* can reflect the taxonomic status, genetic diversity, and population structure genetics.

Methods: Totally, 52 formalin-fixed paraffin embedded (FFPE) tissue samples from patients with histologically confirmed CE were collected from Mazandaran province, Iran in the period of March 1995 to December 2018. All extracted DNAs from (FFPE) tissue samples were subjected to amplify by *polymerase chain reactions* method targeting cytochrome c oxidase subunit 1 (*cox1*) gene. All PCR amplicons were sequenced to phylogenetic analysis and genetic diversity.

Results: Molecular analysis showed that, 50(96.1%) and 2 (3.84%) isolates were identified as G1 and G3 *E. granulosus* genotypes, respectively. DNA sequence analyses indicated a high gene diversity for G1 (Haplotype diversity: 0.830) and G3 genotypes (Hd: 1.00). Based on multiple sequence alignment analyses, 7 (13.46%; G1 genotype) and 2 (3.84%; G3 genotype) new haplotypes were unequivocally identified.

Conclusion: In this study, for the first time, G3 genotype (*buffalo* strain) was identified from two human hydatidosis isolates in the region. Present study strengthens our knowledge about taxonomic status, transmission patterns of *Echinococcus* parasite to human and heterogeneity aspects of this parasite in clinical CE isolates of Northern Iran.

Keywords: Human hydatidosis, *Cox1*, Genetic diversity Phylogeography, Iran

Adverse Relationship between *Ascaris lumbricoides* and Multiple Sclerosis (MS) Prevalence in Isfahan province, Iran

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Background: Multiple Sclerosis (MS) is an immune-mediated demyelinating disease of human central nervous system. There are many scientific evidences indicating that helminthic infections may control autoimmune diseases. So in this work, the relationship between *Ascaris lumbricoides* infection and Multiple sclerosis in Isfahan province of Iran has been investigated.

Material & Method: The prevalence of *Ascaris lumbricoides* infection was investigated in published papers since 1970s to 1990s. On the other hand, the prevalence of MS was collected from published papers during the last decades.

Results: During the 1970s to 1990s, the *Ascariasis lumbricoides* prevalence was dramatically decreased from about 90% to less than 2%. Furthermore, the prevalence of Multiple sclerosis was sharply increased from 5/100000 to 75/100000.

Conclusion: There is an adverse relationship between the prevalence of *Ascaris lumbricoides* infection and Multiple sclerosis disease prevalence in Isfahan province during the last decades.

Keywords: *Ascaris lumbricoides*, Multiple Sclerosis, prevalence, adverse relationship

Molecular Identification of *Taeniahydatigena* in larval Stage (*Cysticercustenuicollis*) Isolates from Sheep and Goat in Mazandaran Province, Northern Iran

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Abstract

Introduction: *Cysticercustenuicollis* is a metacystode of *Taeniahydatigena* is the common diseases in livestock, especially in sheep and goat, with medical and veterinary importance. Therefore, the present molecular study aimed to identify the genotypic of the larval stage of *T. hydatigena* (*C. tenuicollis*) by molecular methods with mitochondrial *cox1* and *12SrRNA* genes isolates from sheep and goat in Mazandaran province, northern Iran.

Materials and methods: This study was performed on 88 samples of *C. tenuicollis* collected from sheep and goat isolates from slaughter houses in the three regions of Mazandaran province during 2014-2015. After collecting specimens DNA was extracted and amplified by using PCR method with specific primers of *cox1* and *12SrRNA* genes. PCR product sequenced and the phylogenetic analysis was done.

Results: The results of the phylogenetic analysis two mitochondrial genes indicated a genetic similarity between samples in livestock isolates from local and import sheep and goats in Mazandaran province. However, exception of 10 isolates from the western regions of Mazandaran province, all *C. tenuicollis* isolates had a similar genetic relationship. Four cases of sheep and goat isolates with the *12SrRNA* gene in the western region were different genetically compared to other samples.

Conclusion: In this study, we observed genetic similarity between the larval stages of *T. hydatigena* in sheep and goat in Mazandaran province. But, there was a genetic difference between the isolates in west regions of the province which, indicating the intra-specific difference in parasite. Future studies required to determine the genetic characteristics of the adult worm.

Keywords: *Taeniahydatigena*, *Cysticercustenuicollis*, Sheep and goat, Northern Iran

Viability of *Trichomonas vaginalis* in Different Types of Transitional Medium

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Background: *Trichomonas vaginalis* remains a common cause of vaginitis worldwide. At the present time, trichomonosis is most frequently diagnosed by wet preparation examination of vaginal fluid. There is no ordinary microscope and we lack the appropriate specialized medium to culture the parasite in most doctors' offices and transformation of parasite in wet vaginal samples. This study was done to create a proper transport medium to keep parasite alive for a long time without negative change in parasite ability to grow and produce.

Methods: From all 168 women referred to Bandar Abbas Health Centers, vaginal swabs were taken and randomized into two equal groups: Saline, Amies Transport Medium with Charcoal and without Charcoal. Those stored at 25°C and those stored at 37°C. After 3, 6, 12, 24, 48 and 72 hours, the morphological, movement and proliferation ability (in Diamond medium) were investigated.

Results: After 72h *T. vaginalis* parasite could keep its production ability in Diamond medium, being in ATM medium without Charcoal in 25°C and 37°C, while, after 48 h, in other medium it changed form in 25°C and 37°C.

Conclusion: The ability of ATM medium without Charcoal to maintain *T. vaginalis* can help diagnosis, prevention and better treatment of trichomoniasis.

Keywords: *Trichomonas vaginalis*, Transport medium, Bandar Abbas

A Survey on Sarcocystosis in Cattle in Northwest of Iran and Molecular Identification of *Sarcocystis* Spp.

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Background: Sarcocystosis is caused by *Sarcocystis*, a coccidian parasite with numerous species affecting a broad range of animals as well as human. Cattle are intermediate host for several species of this parasite transmissible to their definitive hosts including dog, cat, and human. A high rate of cattle infection has been reported from different parts of the world. The present study aimed to investigate about the prevalence of sarcocystosis and molecular identification of the parasite Spp in cattle in East Azarbaijan, Northwest of Iran.

Methods: In the present study, 100 samples from cattle diaphragm and oesophagus were collected from slaughterhouses of East Azarbaijan province, Northwest of Iran. About 50 gr of each sample was microscopically examined for *Sarcocystis* detection using pepsin acid digestion method. The species of the parasite was consequently detected by PCR-RFLP technique. Finally, ten PCR products of the samples were sequenced for further analysis.

Results: Sixty four percent of the diaphragm and oesophagus samples were found to be infected with tissue cysts of *Sarcocystis*. The PCR-RFLP technique showed that all investigated samples were identified as *Sarcocystis cruzi*.

Conclusion: The study showed that the muscle sarcocystosis in cattle in East Azarbaijan province is relatively high. We also found that *Sarcocystis cruzi* is the most common species in cattle in the East Azarbaijan province.

Keywords: Sarcocystosis, PCR-RFLP, East Azarbaijan province, Cattle, Iran

Northern Urban areas of Tehran and phoretic of the fly with *Ornithocheyletia hallae* (Prostigmata: Cheyletidae) a skin mite of *Columba livia* (Aves: Columbidae)

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Objectives: Avian blood-feeding ectoparasite flies mainly belong to the family Hippoboscidae, commonly known as louse flies. The family is known in Iran by single species *Pseudolynchia canariensis*, pigeon fly, capable of transmitting parasitic agent. Also there is a phoretic association between the fly and mites living on the same avian host, using louse flies to disperse.

Methods: During summer 2018, flies were collected using methods like nets and light traps and preserved in 70% ethanol. In the laboratory, ectoparasites were removed using stereomicroscope or sedimentation in water and detergent solution.

Results: According to their morphological characters, flies were identified as *Pseudolynchia canariensis* based on Huston (1984). A mite species was discovered on their bodies which were identified as *Ornithocheyletia hallae* based on Smiley (1970).

reported from different areas before, including South Khorasan, Kermanshah, Shahrekord, Mashhad and Shiraz. *Myialges anchora* was reported previously from Kermanshah, and just few months ago *Ornithocheyletia hallae* was reported for the first time from Iran and Asia in Shiraz and this is the second record of it from Iran and Asia.

Keywords: Tehran, *Pseudolynchia canariensis*, *Ornithocheyletia hallae*, ectoparasite, *Columba livia*

Survey of Cutaneous Leishmaniasis by Microscopic and Molecular Methods in Suspected Hospitalized Patients in Kashan Shahid Beheshti Hospital during 2013-2019

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Introduction: Cutaneous leishmaniasis (CL) is one of the most important health problems in the worldwide, particularly in Mediterranean countries, including Iran. Isfahan province is the most important foci of CL. The aim of this study was determination of CL rate by microscopic and molecular methods in hospitalized patients.

Material and Methods: Ninety-three patients suspected of Cutaneous Leishmaniasis hospitalized in Kashan Shahid Beheshti Hospital during 2013-2019 were examined. The demographic information as well as signs of patients was recorded in SPSS 16. Serosity of wound were collected and stained by 20% Giemsa, also DNA of 28 cases was extracted and PCR performed by KDNA primer for determination of *Leishmania* species. *Leishmania major* and *L. tropica* were diagnosed by observation of 650 and 750 bp in electrophoresis respectively.

Results: 28 out of 93 cases (30.1%) were diagnosed by microscopic and 23 samples of 28 cases (82.1%) were positive by PCR. From these positive cases 65.2% and 34.8% were *L. major* and *L. tropica* respectively. It be mentioned that two negative cases in microscopic method were positive by PCR. The highest rate (47.1%) of CL was reported in 2014. Also the most positive cases (57.1%) were observed in fall season. The ulcer was observed in 46.4% of infected patients.

Conclusion: According to the results of present study, kDNA-PCR is suitable for diagnosis and determination of parasite species, especially in the cases with fewer parasites. In patients that hospitalized with deep and progressive ulcers like this study, PCR is the best method for timely diagnosis and appropriate treatment.

Keywords: Cutaneous Leishmaniasis, PCR, microscopy, KDNA

Detection of *Echinococcus granulosus* infection in dogs in Kermanshah and Lorestan provinces, western Iran using copro-PCR method

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Abstract

Background: *Echinococcus granulosus* is important in the medical and veterinary zoonotic helminth fields in the tropical and subtropical regions in Iran. Dogs (stray and domestic) represent as the most relevant definitive hosts for *E. granulosus* sensu lato infection. The aim of this study was to determine the prevalence of *E. granulosus* infection among stray and domestic dogs using copro-PCR method in the studied region.

Material and Methods: A total of 605 faecal samples of domestic and stray dogs were collected from two different areas in western Iran during August 2014 to February 2017. The samples were examined using formalin-ether concentration method. The microscopically positive samples were screened for parasites by copro-PCR using the two specific primers for the specific diagnosis of taeniid and *E. granulosus* identity.

Results: Totally, 605 fecal samples from stray and domestic dogs were examined for the presence of the parasites by microscopic examination. Taeniid eggs were observed in 17 (9.4%) of stray dogs and 21 (4.9%) of domestic dogs. Of all taeniid-positive specimens, 21 samples (3.5% of all dog specimens) were contaminated according to primers specific for *E. granulosus*.

Conclusion: Data presented in this study revealed that *E. granulosus* is prevalent in the stray and domestic dogs in west of Iran. The findings of the present study are important from epidemiological standpoint and hydatid control programs in the areas.

Keywords: *Taenia* spp., *Echinococcus granulosus*, Stray dog, Domestic dog

Detection of *Toxocara spp.* eggs in Environmental soil Samples of Mazandaran Province, North of Iran

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Background and objectives: Toxocariasis caused by *Toxocara spp.*, which is one of the most important zoonotic diseases. Ingestion of embryonated eggs in contaminated soil is one of the major transmission routes of acquiring the infection. The aim of present study was to estimate the prevalence of *Toxocara spp.* eggs in environmental soil samples of Mazandaran province, North of Iran.

Martials and methods: A total of 256 soil samples were collected from six cities including Sari, Behshahr, Savadkuh, Fereydunkenar, Chalus and Ramsar in Mazandaran province (North of Iran) by simple random selection during April to September 2014. The samples were taken from different sites including: public parks, public places, vegetable gardens, sand heaps and shadow areas near houses. The prepared soil samples were examined by modified sucrose flotation method for detection of *Toxocara spp.* Eggs.

Results: From the examined soil samples, 11 (4.3%) samples were positive for *Toxocara spp.* eggs. The most and least contaminated locations were sand heaps (6.2%) and shadow areas near houses (2.3%), respectively. Fereydunkenar 5/47 (10.6%), Savadkuh 2/38 (5.2%), Sari 2/40 (5%) and Ramsar 2/42 (4.8%) had the highest contaminated soil samples, respectively while Chalus 0/48 and (0%) Behshahr 0/41 (0%) showed the lowest contamination rates.

Conclusions: As the soil of this areas are contaminated with *Toxocara spp.* eggs, the people and especially children might get the contamination during contact with the contaminated soils. This study provides information for local control of toxocariasis in this province as we showed the contamination of public areas

Keywords: *Toxocara*, Eggs, Soil, Public health, Iran

Comparative IgA and IgM Antibodies against *Linguatula serrata* Infestation in Goat by Indirect Enzyme-linked Immunosorbent Assay (ELISA)

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Abstract

Introduction and Objectives: *Linguatula serrata* is a zoonotic parasite causing visceral and nasopharyngeal linguatulosis in humans. Dog and other canines are the main definitive hosts while most herbivores, including ruminants serve as intermediate hosts for linguatulosis. Human rarely become infected as both final and intermediate hosts. The aim of this survey was designing and evaluation of an indirect ELISA test for serodiagnosis of *L.serrata* infestation in goat by using somatic and excretory-secretory (E/S) antigen.

Materials and methods: The E/S antigens were prepared by incubation larvae in MEM media for 24h. Somatic antigens were prepared by sonication (250 nymphs in 10 ml PBS-MEM). Appropriate dilutions of sera, Rabbit anti goat IgA and IgG: HRP, somatic and E/S antigens were determined as 1:20, 1:4000, 1:20 and 1:4 respectively. Evaluation of ELISA was conducted by 97 sera (72 positive and 25 negative). Sensitivity, specificity, accuracy, negative predictive value and positive predictive value for somatic and E/S antigen were calculated.

Results: Sensitivity, specificity, accuracy, positive predictive value and negative predictive value in the Rabbit anti goat IgA were 95.83%, 84%, 92.78%, 95.83% and 87.50% for somatic and those for E/S antigen were 88.88%, 96%, 90.72%, 98.46% and 75% respectively. Those in the Rabbit anti goat IgM were 97.22%, 96%, 96.90%, 98.59% and 92.30% for somatic antigen and those for E/S antigen were 98.61%, 96%, 97.93%, 98.61% and 92.30% respectively.

Conclusion: The Rabbit anti goat IgM and E/S antigens of nymph stage of *L.serrata* are appropriate to determine the seroprevalence linguatulosis.

Keywords: *Linguatula serrata*, ELISA, Antibody, goat

Helminthic Infections in Poultry Kept in Free Range Systems in Piranshahr, Northwest of Iran

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Background and objectives: Indigenous poultry play a notable role as a source of nutrient elements as well as income for people living in the countryside. They get infected to parasites by ingestion of infective parasitic stages present in soil and litter and/or by eating intermediate or transport hosts. Infections with parasitic helminthes may have severe consequences on the host as well as on the production systems as reported by several studies. The current study was carried on to determine infection to gastrointestinal helminthes in native poultry kept in traditional systems in Piranshahr, the northwest of Iran.

Methodology: For this purpose, 85 native poultry from different parts of the study area were randomly selected. The gastrointestinal tracts were accurately inspected for the presence of parasitic helminthes using standard parasitological approaches.

Results: The results indicated that the prevalence of parasitic helminthes was 36.4%. Two nematodes (*Heterakis gallinarum* 21.1%, *Ascaridia galli* 12.9%) and one cestode (*Raillietina sp.* 15.2%) were isolated. Severe infections with *Raillietina sp* were observed.

Discussion and conclusion: Helminthes may obstruct the small intestine and cause death. They can act as vectors and lead to secondary infections, e.g. *E. coli*. Furthermore they have adverse effects on behavior patterns, growth and nutrient utilization of chickens. The data revealed that it is necessary to perform control strategies in order to decrease the helminthic infections in the poultry of the study area.

Keywords: Native poultry, *Heterakis gallinarum*, *Ascaridia galli*, *Raillietina*, Piranshahr.

Parasitic Infestation in Freshwater Fish in Piranshahr, Northwest of Iran

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Background and objectives: Since the human body cannot make significant amounts of some necessary nutrients, fish are an essential part of the diet. It has been proved that parasites have negative impacts on fish health and production. Also, fish can serve as an intermediate, paratenic (transport) or definitive host for various stages of parasites.

Methodology: This study was performed to evaluate the prevalence of fish ectoparasites in natural rivers of Piranshahr, northwest of Iran. Totally, the whole bodies of 120 fish specimen were inspected for ectoparasites. The collected samples were then transferred to laboratory and surveyed using microscope and stereomicroscope.

Results: The results revealed that 47 fish (39.1%) were infested to ectoparasites. The isolated parasites were *Dactylogyrus spp.* (25, 20.8%), *Ichthyophthirius multifiliis* (18, 15%) and *Trichodina* (7, 5.8%). Fourteen fish (11.6%) showed severe infestation along with damaged skin and gills.

Discussion and conclusion: Parasites are a significant stressor affecting fish populations. They can cause fish death, loss of appetite, the slowdown in growth, deterioration of reproductive ability, reduction of resistance to other pathogens as well as marketing with unpleasant views. This research presented basic information about prevalence of ectoparasites in freshwater fish in Piranshahr. More comprehensive studies are needed to gain accurate data on prevalence, species and other important aspects of parasites in fish in the study area.

Keywords: Ectoparasites, Freshwater fish, Piranshahr, Iran

The occurrence of *Ligula intestinalis* plerocercoid Infection in Freshwater Fish in Piranshahr, Northwest of Iran

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Background and objectives: Parasitic agents, in particular cestodes parasites are of the major threats affecting fish health and breeding. They may have negative impacts on fish in terms of growing properly, resulting in weak and powerless fish. One of the important parasitic diseases of fish is ligulosis caused by *Ligula intestinalis* plerocercoids. It infests a range of freshwater fish species as the second intermediate host. The plerocercoid is also infective to a wide range of fish-eating birds, which serve as the final host. It can cause a significant loss in fish industry by making pressure on the organs in the abdominal cavity and importantly, suppressing the development of the gonads.

Methodology: Fifty fish caught by net and hooks in Silveh Dam, Piranshahr were dissected for internal parasites

Results: Accidentally, during the inspection of the fish for internal parasites, severe infections with *L. intestinalis* plerocercoids were observed in six examined fish.

Discussion and conclusion: Since, there are a number of migrant fish-eating birds around the dam, it may be concluded that these birds have transmitted the parasite from infected places to the study area. Fish infected by *L. intestinalis* plerocercoids are not able to swim properly, and their bellies are tumid; furthermore, it can be seen that bellies burst and the parasites get out. Death occurs in severe infections.

Keywords: *Ligula intestinalis*, Plerocercoid, Fish, Piranshahr

Study on Parasitic Contamination of Raw Vegetables in Shoushtar City, Southwest of Iran, during 2018-2019

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Introduction: Regard to important of parasitic infection also raw vegetable as essential source of vitamins and fiber specially Iranians tablecloth arrangement so this study aimed at valuing level of parasitic contamination of fresh vegetables in Shoushtar city, Southwest of Iran, during a years ago.

Material and Methods: The current investigate carried out on 90 sample washing water of vegetable with formal ether sedimentation and Sheather's sugar technique added to wet smear and Ziehl Neelsen Staining.

Results: Generally, Eighty-one percent contamination was reported that Amoeba spp. 41%(30 case) as highest rate of contamination, *Trichomonas* spp. 13.6%(10 case), *Entamoeba coli* 27.3%(20 case), *Giardia* cyst spp. 19.1% (14 case), *Cryptosporidium* spp. 2.3%(2 case), *Blastocystis* spp. 27.3%(20 case), *Strongylous* spp. 3%(3 case) added to kind of ova species respectively including *Trichostrongylus*, *Ascaris*, *Taneaia*, *Hymenolepis*, 1.3% (1 case), 2.7% (2 case), 1.3% (1 case), 2.7% (2 case) and some flagellatae were founded.

Discussion & Conclusion: Based on our results look that it is necessary education to farmers regard to applicant of human and animal fertilizer, suitable washing of vegetables before serving for preventing parasite transmission to body and effective control of infection, operation of sanitation, farming modification however further investigate on sources of irrigation water is needed.

Keywords: Parasitic contamination, Row vegetable, Shoushtar,

The Role of Dogs and Cats as Serious Sources of Parasites for Human

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Background and objectives: No doubt having pet animals has increasingly become popular among people worldwide. Dogs and cats are of the most popular pet animals kept by human. They also can serve as primary hosts for a large number of parasites including endoparasites and ectoparasites which may affect their health and wellbeing. At the beginning of the 21st century, parasitic diseases are still causing a severe burden on animal and human populations in tropical and subtropical regions around the world. It is noteworthy to mention that several dog and cat parasites (e.g., *Toxoplasma gondii*, *Dipylidium caninum*, *Echinococcus granulosus*, *Ancylostoma caninum* and *Toxocara canis*) are important not only from a veterinary perspective but also from a medical standpoint. These parasites can be easily transmitted to human and may severely threaten human health in specific situations.

Discussion and conclusion: Human is infected to *T. gondii* by ingesting sporulated oocytes or tissues cysts. This parasite may cause a serious and occasionally fatal illness in infants, HIV/AIDS patients, and others with weakened immunity. Also, human can be infected by *Toxocara spp.* by accidentally ingesting infective eggs that may be present on the host's fur. When humans ingest infective eggs, diseases like hepatomegaly, myocarditis, respiratory failure and vision problems can result. To prevent the potential risks associated to owning a pet dog or cat, it is fundamental to maintain them in good health and protected from zoonotic pathogens. Veterinary practitioners and medical physicians should work together towards improving the wellbeing and general health of both animals and humans.

Keywords: Parasites, Dog, Cat, Human

Evaluation of Parasitic Contamination in Commonly Consumed Raw Vegetables in Piranshahr, Northwest of Iran

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Background and objectives: It has been reported that regular consumption of raw vegetables which are among the richest sources of vitamins, dietary fiber and minerals, is related to a reduced risk of cardiovascular diseases, stroke and certain cancers. However, ingestion of raw vegetables represented a significant route of transmission of several infectious agents in particular parasites owing to their complex surface and porosity, which unfortunately facilitate pathogen attachment and survival. The aim of the present study was to survey the parasitic contamination in some common raw vegetables in Piranshahr, Iran.

Methodology: Forty vegetable samples were collected from various parts of the study area in order to search for parasites. The samples were concentrated by centrifugation and inspected using common reliable methods including floatation and staining to visualize parasitic stages.

Results: The results showed that 17 samples (42.5%) were infected with at least one parasite. The prevalence of helminthic and protozoan parasites were 12 (30 %) and 9 (22.5 %), respectively. The isolated parasites were *Taenia* spp. (4), *Ascaris* (3), *Trichuris* spp. (2), *Trichostrongylus* spp. (5), *Entamoeba histolytica* (1), *Entamoeba coli* (6) and *Giaedialamblia* (5).

Discussion and conclusion: This study indicated a high contamination of vegetable samples with different parasitic stages. These data emphasize the importance of raw vegetables in threatening public health by transmission of intestinal parasites to humans in the study area. It is essential to wash and clean vegetables in carefully regulated ways before consuming to reduce the risk of the contamination.

Keywords: Parasites, Vegetables, Piranshahr, Iran

Evaluating and Launching the Performance of LAMP Diagnostic Test in the Detection of Toxoplasmosis and Determining the Rate of Contamination Load by Real Time-PCR in Hemodialysis Patients

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Objective: Toxoplasmosis is an infectious disease caused by as a kind of single-celled, intracellular called *Toxoplasma gondii*; despite being asymptomatic in the majority of healthy individuals, it can cause significant complications and bring about mortality in subjects with immune deficiency

Methods: A case-control study was performed on 320 subjects, 160 with hemodialysis and 160 healthy controls as control group in Zahedan, southeast of Iran. Subjects diagnosed diabetes through FBS and A1C diagnostic tests were removed in both groups of control and case. Two blood samples were collected from each individual for conducting ELISA tests and molecular experiments. DNA molecular samples were extracted and, then, tested, using specific primers, using Nested-PCR, Real Time-PCR and LAMP (loop-mediated isothermal amplification) tests based on the RE gene.

Results: Of 160 blood samples collected from hemodialysis patients, 75 turned out to be positive through ELISA method for TB infection; 31.87% (51 out of 160) and 33.75% of the subjects were diagnosed *Toxoplasma* positive based on RE gene using Nested-PCR technique and Lamp in order.

Conclusions: The result of the present study indicates that Lamp and Nested-PCR based on the RE gene sequence molecular techniques are more efficient than serologic methods in detecting active and reactive cases of toxoplasmosis in hemodialysis patients.

Keywords: *Toxoplasma gondii*, hemodialysis patients, LAMP, Nested-PCR

Comparison of Microscopy Method and pfLDH Assay for Determining the Amount of Growth Inhibition of *Plasmodium falciparum* Parasite after Exposure to Chloroquine in Vitro

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Introduction: The major method for diagnosis of malaria in Iran is the examination of Giemsa-stained peripheral blood smears, but the accuracy of the results in this method depends largely on the skill of the laboratory technician and the test conditions. The present study aimed to compare microscopy method and *Plasmodium falciparum* lactate dehydrogenase assay for determining the amount of growth inhibition of *Plasmodium falciparum* parasite after exposure to chloroquine *in vitro*, with regard to recent reports of mistakes in microscopic diagnosis of malaria in the malaria control laboratories.

Materials and Methods: In this experimental study, first, *Plasmodium falciparum* parasites were cultured in flasks containing RPMI1640 medium. After proper growth of the parasites in the culture medium, 150 µl was removed from each flask containing the parasites in the ring stage and transferred to wells of two separate microplates. Then, 50 µl of different concentrations of chloroquine was added to each microplate and incubated at 37 ° C for 48 hours. Thin blood smears were prepared from each well in one of the microplates and stained with Geimsa and counted microscopically. The other microplate was tested by *Plasmodium falciparum* lactate dehydrogenase assay.

Results: The results showed that the degree of inhibitory effect of chloroquine, at a common dose of 14 nmol, using microscopy method and pfLDH assay were 98% and 96%, respectively. Also, these results indicated a 45% inhibitory effect at a dose of 7 nmol of chloroquine using both methods.

Conclusion: According to statistical analysis performed on the results of the present study, reported differences between the two methods (microscopy and pfLDH) were not statistically significant ($p > 0.05$). Therefore, if specialist staff are employed for the microscopic diagnosis of *Plasmodium* parasites, this method is also useful, as the enzyme method, for comparing the parasitemia in both the control group and the treatment group.

Keywords: *Plasmodium falciparum*, pfLDH, Parasitemia, Giemsa

Report of the Prevalence of *Trichomonas vaginalis* in Iran (A Review Study)

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Introduction: Trichomoniasis is a sexually transmitted infection in the genital-urinary tract. Symptoms of acute trichomoniasis often include yellow vaginal discharge, urethral itching or burning and frequent urination. Transmission of the disease occurs through sexual intercourse, especially by men with and without clinical signs. We aimed to investigate the prevalence of trichomoniasis in Iran with regard to the health importance of this infection and the controversies in its prevalence.

Methods: In the present study, keywords *Trichomonas*, Prevalence, Iran indexed in Magiran, SID, Iranmedex, IRANDOC, SCOPUS, PUBMED, ISC, and Google scholar databases were used. Out of the searched articles, only the articles related to the prevalence of trichomoniasis in different regions of Iran were reported and the remaining articles were removed.

Results: Investigation of *Trichomonas vaginalis* infection in different regions of Iran showed that the prevalence of the parasite is dependent on culture, health, general information level and general condition of the individual in terms of health and history of drug use.

Analysis of the data from different papers showed that the prevalence of *Trichomonas* in Iran is 0.8 to 11.3 percent, which it has decreased than ever before. However, it should be noted that the degree of affection in high risk individuals has also been reported up to 30%.

Conclusion: Comparison of the results from various papers indicates that the prevalence of Trichomoniasis in Iran today is lower than its prevalence in previous years. Increasing health and changing people's awareness and lifestyle can be involved in this decline.

Keywords: *Trichomonas vaginalis*, Prevalence, Iran.

In Vitro Anti-Leishmanial Effects of Tavaborole on *Leishmania major*

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Background and objectives: *Leishmania major* is the agent of a zoonotic disease named cutaneous leishmaniasis that is endemic in Iran. Tavaborole is a topical antifungal medication for the treatment of onychomycosis, a fungal infection of the nail and nail bed. The aim of this study is to evaluate anti-leishmanial effects of tavaborole on *Leishmania major*.

Methods: The promastigotes and amastigotes of this parasite were treated with the tavaborole in several concentrations at 72 hours and the drug inhibitory concentration of 50% (IC₅₀) was assessed.

Results: The highest effect on promastigotes was in concentration of 658 μ M with 58.4% death rate. Regarding amastigotes, the highest effect was in concentration of 658 μ M with 57% death rate. IC₅₀ for promastigotes was 374 μ M. Likewise, IC₅₀ for amastigotes was 498 μ M.

Conclusion: According to the results, it indicated that tavaborole had not the selectivity function on the parasite and has toxicity effect on the cell. Further studies are needed to clarify the toxicity effect of this drug in vivo.

Keywords: *Leishmania major*, tavaborole, in vitro

Anti-Leishmanial Effects of Efinaconazole on *Leishmania major* in Vitro

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Background and objectives: Leishmaniasis is a disease caused by an intracellular parasitic protozoan genus *Leishmania* transmitted by the bite of a female phlebotomine sand fly. Leishmaniasis is endemic in 88 countries throughout Africa, Asia, Europe, and North and South America. Efinaconazole (10%) topical solution is a new triazole recently approved for the treatment of onychomycosis. It inhibits fungal lanosterol 14 α -demethylase in the ergosterol biosynthesis pathway. The aim of this study is to evaluate anti-leishmanial effects of efinaconazole on *Leishmania major* in vitro.

Methods: The promastigotes and amastigotes of this parasite were treated with the efinaconazole in several concentrations at 72 hours and the drug inhibitory concentration of 50% (IC₅₀) was assessed.

Results: The highest effect on promastigotes was in concentration of 143 μ M with 58% death rate. Regarding amastigotes, the highest effect was in concentration of 143 μ M with 62% death rate. IC₅₀ for promastigotes was 113.7 μ M. Likewise, IC₅₀ for amastigotes was 89 μ M.

Conclusion: According to the results, it indicated that efinaconazole affected on parasites and showed selectivity index and no toxicity effect was observed on cell alone. These effects show that this drug can be as a candidate for treatment of cutaneous leishmaniasis.

Keywords: *Leishmania major*, efinaconazole, in vitro

Seroprevalence of Toxoplasmosis in General Population and Pregnant women in the West of Iran

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Background and objectives: It is estimated that one-third of the world's population is infected with *Toxoplasma gondii*. The purpose of this study was to evaluate the latest status of toxoplasmosis seroprevalence in the general population and pregnant women in the west of Iran.

Methods: This retrospective cross-sectional study was conducted in 2018. Accordingly, data associated with serodiagnosis of toxoplasmosis, age, sex, anti-toxoplasmosis IgG and IgM, and pregnancy status in women were collected from health centers of Kermanshah City, the west of Iran, during 2016-2017.

Results: In total, 1228 people referred to the health centers in Kermanshah City. Of 1228 people, 359 (29.23%) individuals were seropositive for toxoplasmosis, of them 294 (81.89%) individuals were seropositive only for IgG, and 65 (18.11%) individuals were both IgG and IgM seropositive. The seropositivity in men was 29.3% (n = 63), in women was 29.2% (n = 296), and in pregnant women was 25.9% (n = 44).

Conclusion: This study showed that the prevalence of this disease in the west of Iran has been decreased in comparison with the previous studies. Therefore, regular epidemiological studies of in different regions seem to be necessary in order to conclude on the decrease or increase trend of this disease in an area.

Keywords: General population, pregnant women, Seroprevalence of toxoplasmosis, West of Iran.

The Effects of Garlic and its Derivatives on Human Parasitic Protozoa: A Systematic Review

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Background and objectives: Garlic is one of the important herbs used in medicine and is as a valuable medicinal herb in various cultures.

Methods: The search was performed in five English databases, including Scopus, PubMed, Web of Science, Embase, and Google Scholar up to December 2018. Studies in any language were entered in the searching step if they had an English abstract. The words and terms were used as syntax with specific tags of each database.

Results: Of 2146 studies, 25 were eligible to be reviewed. The findings showed that the most performed studies (40%; n = 10) were on *Leishmania* species. In addition, 12 studies (48%) were carried out on garlic bulb, followed by four studies (16%) on allicin compound. The mostly used solvent for the preparation of garlic extract was distilled water (36%; n = 9), followed by methanol (16%; n = 4). Moreover, seven studies (28%) were performed in vivo, 11 studies (44%) in vitro, and seven studies (28%) both in vivo and in vitro.

Conclusion: All of the reviewed studies concluded that garlic compounds and its derivatives have potent anti-parasitic effects without the least side effects such as toxicity and can be an appropriate alternative or complement to synthetic drugs for preventing and treatment of parasitic protozoa infections. Moreover, these compounds are able to eliminate the symptoms of the infectious parasitic diseases.

Keywords: garlic, protozoa, systematic review

Genotyping of *Giardia lamblia* Isolates from Human in Zahedan, Southeastern Iran

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Background: *Giardia lamblia* is considered as one of the major enteric protozoa, which infect many mammalian hosts, including domestic and wild animals, as well as humans. Thus far, genetic researches using triose phosphate isomerase (tpi) and the glutamate dehydrogenase (gdh) locus have been identified eight major groups of this parasite in various hosts. The present study was conducted to identify the molecular genotypes of *G. lamblia*, which infected people in the city of Zahedan, Southeastern Iran. This study provides the first data on the genotypes of *G. lamblia* on humans in Zahedan, Southeastern Iran.

Methods: Samples of stool were obtained from the people infected with *Giardia*. Making use of glass beads, a commercial kit, as well as thawing and freezing methods, from the stool samples, DNA was extracted. Addition, utilizing the Semi-nested PCR, the 432bp fragment of glutamate dehydrogenase (gdh) locus was amplified. Using RsaI and BSPII restriction enzymes, the RFLP-PCR (restriction fragment length polymorphism-polymerase chain reaction) was carried out to determine the genotypes of the samples infected with *Giardia*.

Results: PCR-RFLP demonstrated 77.5% of the isolates (n=62) as genotype AII and 15% of the isolates (n=12) as genotype BIII. In 7.5% of the isolates (n=6), the mixed genotype (BIII and AII) was identified.

Conclusion: The findings of the present study demonstrated that genotype AII led to the majority of human infections in the studied area. According to the obtained results, *Giardia* infection could have an anthroponotic origin in the city of Zahedan, Southeastern Iran.

Is *Toxoplasma gondii* Related to Schizophrenia?

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Background and objectives: Due to the high affinity of *Toxoplasma gondii* to the nervous tissue (predominantly the glia cells–astrocytes) and the established association with inborn cerebral disorders, many researchers have been interested in studying on probable associations between the parasite infection and severe psychiatric disorders such as schizophrenia. No unique causal agent for schizophrenia has been detected, so far. However, there are some factors including genetic predisposition, neurodevelopmental disturbances and environmental factors e.g. infectious agents were shown to be positively correlated with schizophrenia. *T. gondii* has been considered as a main candidate for a variety of reasons; (i) many studies have reported that individuals with schizophrenia, compared to controls, have a higher prevalence of antibodies to *T. gondii*, (ii) epidemiologically, there are many similarities between toxoplasmosis and schizophrenia, (iii) antipsychotic drugs known to be effective in schizophrenia also inhibit some parasites, including *T. gondii*, and (iv) *Toxoplasma* has been shown to induce elevated levels of dopamine in experimentally infected animals (elevated dopamine is commonly seen in individuals with schizophrenia). However, the above-mentioned reasons are not sufficient to introduce *T. gondii* infection as a definitive agent of schizophrenia because some studies have reported no relation between the infection and schizophrenia.

Discussion and conclusion: A number of questions remain concerning a role for *Toxoplasma* in the aetiology of schizophrenia, including the roles of strain variation, the timing and source of infection, and the role of host genes in determining disease susceptibility. More comprehensive researches are needed to have a better understanding regarding this association.

Keywords: *Toxoplasma gondii*, Schizophrenia, Psychiatric disorders.

Prevalence of Intestinal Parasitic Infections in Referred Individuals to Amir Almomenin Hospital of Gerash City, Iran, 2017-2018

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Background and Objectives: Despite significant promotion in the health care across the world, intestinal parasitic infections are one of the most important and common health problems, especially in developing countries. The aim of this study was to determine the prevalence of parasitic infections in Gerash city (Fars province, Iran)

Materials and Methods: This cross-sectional study was conducted on stools specimens people referred individuals to Amiralalmomenin hospital of Gerash, from March 2017 to March 2018. Collected stool samples were assessed using direct wet mount and formalin-detergent concentration methods.

Results: Out of 5100 stool samples, 172 cases (3.3%) were found to be infected with intestinal parasites. *Blastocystis hominis* (126 cases, 73.3%) had the most frequency. The maximum infection rate (70 cases, 40.7%) observed in the age group of 30-50 years. The overall prevalence of parasitic infections was more in male (98 cases, 57%) than female (74 cases, 43%).

Conclusion: According to the results, the prevalence of intestinal protozoa is higher than worm's infections. Generally, the results show the relative prevalence of intestinal parasitic infections in Gerash city.

Keywords: Intestinal parasitic, Prevalence, Gerash city

Synthesis of a New Peptide Derived from *Fasciola gigantica* for the Serodiagnosis of Fascioliasis

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Background: Fascioliasis is an endemic parasitic disease causes considerable economic impact in Iran and many regions worldwide. Cathepsin L is a member of the endopeptidase proteases which is widely expressed in *Fasciola* species. The purpose of this study was to evaluate two synthetic peptides from *F. gigantica* cathepsin L1 for serodiagnosis of *Fasciola* infection.

Methods: The potential diagnostic value of surface epitopes of cathepsin L1 was assessed using ELISA and its sensitivity and specificity was determined in performance of ELISA. The bioinformatics tools were used to select two appropriate epitopes of *Fasciola* cathepsin L1 as a synthetic antigen. Their diagnostic values were evaluated by two methods of indirect ELISA and Dot-Blot. The findings showed that the first peptide at 1:400 dilution and the second peptide at 1:100 dilution had the best results and the best concentration of antigens was introduced at 4 µg/ml. Moreover, 156 sera samples were analyzed by both peptides using ELISA method including fascioliasis sera, other parasitic sera, as well as negative serums.

Results: The sensitivity of the peptide 1-ELISA and peptide 2-ELISA for the diagnosis of various cases was 100%. The specificity of the first peptide was 87.3% and its efficacy was calculated to be 93.65%. Moreover, the specificity and efficacy of the second peptide were 79% and 89.5%, respectively. The positive predictive values of the first and second peptides were obtained to be 86.27% and 79.27% respectively, and the negative predictive value of both peptides was calculated as 100%.

Conclusion: The results of this study indicated that the peptide 1 from cathepsin L1 may be used as an appropriate antigen for the diagnosis of fascioliasis.

Keywords: Fascioliasis, *F. gigantica*, cathepsin L1, Protein, Diagnos

Identification of Immunoreactive Proteins in Secretions of *Leishmania infantum* promastigotes Using Immunoproteomics Approach

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Objective: In Mediterranean regions, *Leishmania infantum* is the main cause of visceral leishmaniasis (VL). The secreted antigens of *Leishmania* promastigotes are considered as strong stimulants of the immune system. Since immunoproteomics approach can facilitate the introduction of new vaccine targets, our study attempts to introduce probable new candidates for VL vaccine in secretions of *L. infantum* promastigotes.

Methods: The secretions were prepared from 6×10^9 *L. infantum* promastigotes in RPMI-1640 serum-free media during a period of 72 hours. After deionization and lyophilization, two-dimensional gel electrophoresis was employed for protein separation followed by Western blotting. Thirteen common and repeatable immunodominant spots were identified by Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

Results: From 13 analyzed immunodominant spots, 9 proteins including iron superoxide dismutase, hslvu, phospholipase C, immune inhibitor A, chitin-binding protein and a single peptide match to chain A crystal structure of selenomethionine were identified in the secretions of *L. infantum* promastigotes.

Conclusions: It seems that most of the identified proteins play a role in the metabolism pathways, survival, and pathogenicity of *Leishmania* parasites. For further evaluation of the immunostimulatory property of these proteins, more experiments such as Western blotting and in vivo assays are needed to introduce them as candidates for developing VL vaccines.

Key-words: *Leishmania infantum*, Immunoproteomics, Secretion

***Balamuthia mandrillaris* in Environmental (water, soil and dust) Samples of Isfahan City, Central Iran 2019 (First report)**

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Introduction and objects: *Balamuthia mandrillaris* is a free-living amoeba and an opportunistic pathogen which lives near human settlements. It causes Balamuthia Amoebic Encephalitis (BAE) that is usually fatal. It has already been isolated from environmental samples. Most of infections have originated in warm areas; however it can survive in other climates as well. Our object was to investigate of *Balamuthia manderillaris* frequency in environmental samples of Isfahan city central Iran for first time.

Material and methods: 96 environmental samples (32 water, 31 soil and 33dust) were collected from different areas in Isfahan city central Iran. The samples were cultured in 2% non-nutrient agar (NNA) medium. Positive results were then followed by staining and microscopic studies.

Results: In the present study, we successfully isolated *B. mandrillaris* in 15 (15.63%) of samples over all. The positive samples distributed as: soil 7(22.58%), water 5(15.63%) and dust 3(9.09%) between related samples.

Discussion and conclusion: Our results confirm that *B. mandrillaris* able to spread in Isfahan climatic region and can cause BAE infection. We recommend that BAE would be considered as a probable etiologic agent for some encephalitis cases by clinicians.

Key word: *Balamuthia mandrillaris*, Environmental samples, Isfahan, Iran

Anti-leishmanial Activity of Saponin-Rich Extract of *Allium giganteum* on Promastigotes of *leishmania major*

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Abstract

Background and purposes: Leishmaniasis is a major health problem worldwide. Serious side effects, the lack of effective vaccine and emerging of resistance to anti-*Leishmania* drugs, promote the assessment for new therapeutic compound almost in plants. The aim of this study was to evaluate the effect of *Allium giganteum* saponin rich compounds on promastigotes of *leishmania major*.

Material and methods: The chloroform-methanol extract was concentrated under vacuum and fractionated by MPLC on RP-11 column. The saponin-riched fraction was detected by TLC and HNMR analyses and evaluated for Leishmanicidal activity indifferent concentrations and treatment times using MTT and ELISA methods.

Results: The strongest anti- *Leishmania* activity was seen at concentration of 222 µg/ml that killed all available parasites in 22 hours. The mean of IC₅₀±1SD for 24, 48 and 72 hours after exposure was 90.01±13.42, 75.31±5.12 and 62.85±3.96 µg/ml of extract respectively.

Discussion and conclusion: The results of this study showed significant Leishmaniacidal effects of saponins rich extracts of *Allium giganteum* on promastigote in vitro. Complementary studies on effects of saponin-rich extract on amastigote in macrophages, and animal models and isolated compounds in the active compounds were recommended.

Keywords: Leshmaniacidal, *Allium giganteum*, *Leishmania major*, Promastigote

Investigating the invitro effects of some cell membrane stabilizer drugs on *Leishmania major*

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Background: The first line treatment against cutaneous leishmaniasis is meglumine antimoniate. About this drug in addition to being expensive ,reported side effects and enhance resistance development. The Leishmanicidal effects of Ketotifen , Cromolyn sodium and Cinnarizin, were investigated on standard strains of *Leishmania major*.

Materials/methods: *Leishmania major* parasites were first cultured then drug concentrations 5, 10, 15 and 20 µg/ml were added to *Leishmania major* culture at 24, 48 and 72 hours intervals.

MTT assays were performed. *Leishmania major* promastigotes were augmented to the in vitro cultured macrophages and then incubated for 72 hours. IC50 were ascertained and flowcytometry performed. The results were compared with that of Glucantime. Each test was repeated trice.

Results: IC50 values of Ketotifen, Cromolyn sodium and Cinnarizine at 20 µg/ml concentrations after 72 hours were calculated to be 2.04, 17.67 and 34.76 µg/ml for promastigotes and 0.12 ,14.79 and 23.73 µg/ml for amastigotes respectively. The results of MTT assays showed 20% ,35% and 48 % promastigote viability after 72 hours exposure to Ketotifen ,Cromolyn sodium and Cinnarizine at 20 µg/ml concentration. Apoptosis in Ketotifen ,Cromolyn sodium and Cinnarizine were quantified to be 11.52% ,9.96% and 13.66 % in promastigotes and 99.5%, 98.6% and 98.7% in amastigote infected macrophages. Results showed 8%, 17% and 53% toxicity of CNZ, KET and Cromolyn sodium respectively after 72 hours at 20 µg/ml concentration. All treatments produced significantly different results from the control groups (P<0.05).

Conclusions: The results indicated early and late apoptotic and Leishmanicidal effects of the drugs.

Keywords: Ketotifen, Cromolyn sodium, Cinnarizine, MTT, Apoptosis

Evaluation of Immune Response Induced by Nanoliposomes Containing the Imiquimodadjuvant against Leishmaniasis

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ABSTRACT

Background & Purpose: Leishmaniasis, is a parasitic vector-borne disease, which poses a significant public health threat globally (1). In this study, we examined the properties of the TLR7 agonist imiquimod, a vaccine adjuvant, making use of a live model of infection where the immune reactions could be identified prior to and following the challenge of infection.

Materials & Methods: The liposomes of EPC containing the imiquimod adjuvant were provided and identified for protein concentration, surface charge and particle size (2). Vaccination was done using the soluble *Leishmania* antigen (SLA) as a first-generation vaccine's model in the liposomal state to vaccinate BALB/c mice against the challenge of *Leishmania major*(3). BALB/c mice were vaccinated subcutaneously, three times at a two-week interval (4). Parasite burden, footpad swelling, IgGisotype, as well as the level of IL-4 and IFN- γ were assessed as the protection criteria (5).

Results: The group of mice vaccinated by Lip+Imiquimod+SLA demonstrated a lower amount of footpad swelling and parasite burden than the buffer group. In addition, the greatest amount of IFN- γ and the smallest amount of IL-4 production were noticed in the splenocytes of the mice vaccinated by the formulation of Lip+Imiquimod+SLA.

Conclusion: Badiie et al. research shows that, liposomes provided using the DPPC or DSPC-containing antigen of rgp63 demonstrated more entrapment efficacy and were capable of stimulating a better Th1 response than liposomes provided using EPC-containing rgp63, as a recombinant antigen that prompts a Th2 kind of immune reaction(6). Over results show that imiquimod added to the formulation of liposomes is able to modulate the immune reaction of the BALB/c mice vaccinated preferably to a Th1 reaction rather than a Th2 reaction; it can also lead to partial protection against the challenge of *Leishmania*.

Keywords: Leishmaniasis, Imiquimod, Liposome, Immune response

Molecular Detection of *Toxoplasma gondii* in Cancer Patients in Zahedan city, Sistan and Baluchestan Province, Iran, 2018

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Abstract

Background & Purpose: Toxoplasmosis is a disease caused by the infection as a kind of obligate intracellular and protozoan parasite(1).Contamination with protozoan is important in some people, such as pregnant women and those with the immune deficiency (2). A quick and accurate diagnosis of toxoplasma infection is very helpful in controlling, preventing and treating disease progression in societies at high risk. In this study, the PCR technique was compared with ELISA method by using the RE gene in detecting toxoplasmosis in cancer patients (3, 4).

Materials & Methods: This experimental study was carried out on 154 patients with cancer, who admitted to Imam Ali Hospital in Zahedan. Two blood samples were taken from the patient for the ELISA test and molecular test. Simultaneous with diagnosis of people with Toxoplasmosis by ELISA serology, the whole DNA Blood Samples were extracted and primers were then selected and the PCR technique was performed using the RE gene in the detection of toxoplasmosis.

Results: Among 154 blood samples of patients with cancer, 39 individuals (25.3%) were diagnosed positive for the *Toxoplasma gondii* antibodies by the ELISA technique. After performing the PCR technique, 48 (31.1%) samples were diagnosed positive for the RE-gene among 154 cancer patient samples based on the RE gene sequence, of which 26 common samples (54.1%) were positive by the ELISA technique.

Conclusion: Fallahi et al to detect *Toxoplasma gondii* in children with leukomycosis, using Nested-PCR based on two RE and B1 genes, RE was found to be more sensitive to B1 gene (5).The results of this study indicate that PCR technique based on the RE gene sequence is much more accurate than ELISA method for detecting *Toxoplasma gondii* DNA in the active cases of disease.

Keywords: *Toxoplasma gondii*, Cancer, PCR

In Vitro Scolicidal Activity of Synthesized Albendazole Nanocrystals *Echinococcus granulosus sensu stricto*

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Abstract

Background: the larval stage of the cestoda *Echinococcus granulosus* caused hydatidosis in both humans and domestic animals. Benzimidazole is a compound which is used for the treatment of this disease in human.

Objectives: The aim of this study was to investigate the effect of albendazole and Nano-albendazole nanocrystals (ABZ-NC) on the viability of protoscoleces in vitro.

Method: After collection of hydatid cyst livers from sheep slaughtered in the slaughterhouse, the study was carried on the fertile cysts. The protoscoleces were evacuated, washed and their viability was examined. The certain volumes of protoscoleces were poured into falcon tubes containing culture media RPMI-1640 and ABZ and ABZ-NC solutions were added with final concentration 1 µg/ml. The protoscoleces were recollected after specific time interval and their viabilities were examined by staining with Eosin under an optical microscope. To determine the genotype of the parasite, the DNA was isolated from the protoscoleces and amplified using a pair of primers JB3 / JB4.5 and ultimately, the nucleotides derived from the PCR product were determined.

Results: The results showed that ABZ-NC is more effective in removing protoscoleces. The group in which ABZ-NC were used, the amount of viability of protoscoleces came to zero after 17 days, while the viability of protoscoleces in the ABZ group lasted about 28 days ($p < 0.05$). The nucleotide sequence showed that the genotype of the protoscoleces used in this study was *Echinococcus granulosus sensu stricto*.

Conclusion: According to the results of this research, ABZ-NC can be used as a suitable drug in the elimination of hydatid cyst protoscoleces. So its usage in hydatid cyst surgery as a scolical combination requires further studies.

Keywords: Hydatid cyst, Protoscolex, Albendazole, Nanocrystals, Culture media, *Echinococcus granulosus*.

In Vitro Scolicidal Effects of *Artemisia vulgaris* and *Calendula officinalis* Extracts

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Background: Hydatid cyst is the larval stage of *Echinococcus granulosus* tapeworm. It is endemic and is still an important economic and public health concern in some parts of the world, especially in the Middle East. Main treatment options for CE is surgery and removing the cyst. Surgical treatment involves the use of various scolicidal agents to kill infective *E. granulosus* protoscolices that may disseminate into the peritoneal. So in this work sciolicidal potential of *Artemisia vulgaris* and *Calendula officinalis* have been investigated.

Method: Two plant extracts including: *Artemisia vulgaris*, *Calendula officinalis* were prepared. Then live protoscolices were treated with these extracts at different concentrations and times. The viability of protoscolices was tested using 0.1% aqueous solution of eosin stain.

Results: *Artemisia vulgaris* extract killed 100% of treated protoscolices following 5minutes of exposure. *Calendula officinalis* extract killed 80% and 100% of protoscolices following 5 and 10 minutes exposure respectively.

Conclusion: *Artemisia vulgaris* and *Calendula officinalis* Extracts have a very high protoscolicidal activities. Further work is recommended to prepare them for use in human hydatid cyst surgery.

Keywords: *Artemisia vulgaris*, *Calendula officinalis*, Scolicidal, Hydatid cyst

Genotyping and Phylogenetic Analysis of *Plasmodium vivax* circumsporozoite Protein (*pvcsp*) Gene of Clinical Isolates in South-Eastern Iran

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Abstract

Background: Circumsporozoite protein (CSP) is one of the most important surface sporozoite antigens in malaria, which is recently considered as a candidate for vaccination. Considering the importance of CSP, this study was conducted to investigate the polymorphism and genetic diversity of *Pvcsp* in the southeastern region of Iran.

Methods: To investigate polymorphism and genetic diversity, 20 blood samples were collected from patients with *Plasmodium vivax*, then DNA was extracted and amplified using partial sequence of CSP gene. Polymerase chain reaction (PCR) products were sequenced and compared to a sequences from genomic databases using BLAST. Genetic evaluation and phylogenetic analysis were performed using MEGA7 and DnaSP5 software's on 38 sequences include 20 sequences of our study and 18 sequences of Gene Bank.

Results: The results showed that 11 isolates were VK210 genotype and 9 isolates contained VK247. The result of variable segregation nucleotide site indicated that the differentiation of sequences in CSP were 25.67% in our 20 samples which is less than the 38 samples with a value of 26.67%. Comparing the ratio of dN/dS regions in the CSP gene indicates that the CSP varies more synonymously and amino acid has lower variation. Out of 38 samples, 35 unique haplotypes were identified based on 1042 nucleotide sequences in CSP, showing a variation percentage of 99.4%.

Conclusion: The Tajima D analyses showed that CSP gene in *P. vivax* had a positive number in the total analyzed sequences, which means that the *P. vivax* mutations are in order to select positive evolution.

Keywords: *Plasmodium vivax*, Circumsporozoite protein, Genotyping, Phylogenetic analysis, Iran

Epidemiological, Clinical and Paraclinical Study of Hydatidosis in Isfahan AL Zahra Educational Medical Center during 2007-2016

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Background: Hydatidosis is a zoonosis diseases and an important public health problem in many areas of the world and Iran. Sufficient epidemiological information is required for the taking care, monitoring and effective control of the disease. In the present study, we evaluated epidemiological, clinical and paraclinical characteristics of hydatidosis at one University Medical Centers in Isfahan over a 9-year period.

Methods: This is a descriptive cross-sectional study conducted in patients with hydatid cysts who were hospitalized in Isfahan AL Zahra hospital during the years 2007-2016. The information was collected from the hospital medical records center that include age, gender, job, organ involvement, the number and size of cysts. Also clinical symptoms, and paraclinical information such as CT Scan, MRI, ultrasound, pathological diagnosis were collected.

Findings: Overall, 698 patients, 352 (50.4%) male and 346 (49.6%) were female, who 647 patients were diagnosed as having hydatid cyst by clinical and paraclinical findings, with pathologic documentation. Patients' age ranged from 3 to 87 years, and the peak prevalence of the disease was between 20 and 40 (39% of cases). The involvement of liver was the most frequent 64.8% and most cases of liver cysts (54.2%) were in the right lobe.

Conclusion: The results showed that some cases of hydatid cysts were not detected before surgery in the usual way or, on the contrary, cases of cysts were not hydatid after surgery. This suggests that hydatid cyst diagnosis requires more reliable and accurate diagnostic methods.

Keywords: Hydatidosis, Epidemiology, Surgery, Isfahan

Apoptosis Genes Expression in Bovine Lymphocytes in Response To host Fertile and Unfertile Hydatid Cyst Fluid

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Backgrounds: Hydatid cyst is an infection with global distribution that caused by the larval stage of the tapeworm of *Echinococcus*. The long-term survival of the hydatid in the host shows the parasite has advanced highly effective strategies for escaping the host defense. Deaths caused by parasitic infections, it is often due to tissue damages that the result of a host cell death that known as apoptosis. So it is important to know the process and role of apoptosis that is created or controlled by parasite. Investigation of cytotoxicity effect, induction of apoptosis and mechanism of induction of apoptosis of cattle hydatid liquid on bovine lymphocyte cells as efficient cells of immunity

Methods: In this study, the cytotoxicity effect of bovine hydatid fluid (HF) on lymphocyte cells was investigated as effective immune cells against *Echinococcus* species by MTS method. Then the expression means of caspase-3, Bax and Bcl-2 genes in bovine lymphocytes treated/untreated was determined with fertile and unfertile hydatid cyst fluid using Real Time PCR method.

Results: The viability mean of lymphocytes was significantly lower in the fertile HF treated lymphocytes compared to both unfertile HF-treated lymphocytes and cell control. Bax gene expression was significantly ($P=0.046$) higher in the fertile HF-treated lymphocytes compared to both unfertile HF-treated lymphocytes and cell control. Although, Caspase 3 was higher in this group, the difference was not significant. Also, expression of Bcl-2 gene in fertile fluid treated lymphocytes was found to be lower than that of unfertile and control.

Conclusion: Present study indicate that hydatid cyst fluid molecules can probably induce apoptosis in immune cells in vitro and the parasite ability to stay alive for long time at the host by controlling the host immune response from the apoptosis pathway.

Keywords: Cytotoxicity, Apoptosis, Bax, caspase 3, Bcl2

Reaction of Hydatid Cyst Wall Antisera with Human Breast Cancer Tissues

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Background: Parasites and cancers have a number of properties in common. As an example there are some share antigens between cancers and parasites. So in this work, reaction of anti-hydatid cyst antisera with human breast cancer tissues has been investigated.

Method: Spare breast cancer tissues smears were collected from pathology lab in Alzahra hospital, Isfahan, Iran. The reaction of the tissue with parasite antisera was investigated using immunohistochemistry technique.

Results: Anti hydatid cyst wall antiserum reacted with breast cancer tissues. However normal tissues had faint reaction.

Conclusion: Anti hydatid cyst wall antisera selectively reacted with human breast cancer tissues.

Keywords: Hydatid cyst, Breast cancer, Antiserum, Immunohistochemistry, Parasite.

Common voltage-gated sodium channel mutation in head louse at North-Western Iran

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Objectives: *Pediculus humanus capitis* is an ectoparasitic insect can cause intense scalp itching, secondary bacterial infections, and social stigma. Recently we have report six novel mutations to be located in the IIS1-2 extracellular and loop and IIS5 of the α -subunit in head louse.

Materials and Methods: In the current study, the same analysis was carried out on head louse samples collected from Azarbaijan-Gharbi and Azarbaijan-Sharghi provinces in north-western Iran.

Results: Sequence similarity within specimens collected from Azarbaijan-Gharbi was 100% while it was 99% in Azerbaijan-Sharghi. The variation was because of a silent transition (T/C) in 34 positions. Amino acid sequence similarity was 100% between both provinces. H813P mutation was present in all sequences.

Discussion: Therefore, the presence of a novel mutation in the sodium channel is likely to be the reason for the frequent use of pyrethroid insecticides due to treatment failure against lice. Still, further studies are required to evaluate the prevalence of the *kdr*-like mutant allele for monitoring of insecticide resistance and the management of head lice in other provinces of Iran.

Keywords: head louse, novel voltage-gated sodium channel mutation, knockdown resistance, Iran.

Characterization of acetylcholinesterase gene in *Musca domestica* from Iran

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Objectives: The house fly (*Musca domestica*) is one of the most important health, veterinary and economic pests. Today insecticides such as organophosphate and carbamate are widely used to housefly control. The continued use of these insecticides causes resistance selection. Resistance is associated with a number of mutations in the acetylcholinesterase gene. We investigate the sequence of acetylcholinesterase gene of houseflies.

Materials and Methods: The DNA was extracted using YTA kit and Ace fragment amplified with specific primers in houseflies collected from Azerbaijan-Gharbi, Guilan and Ardebil provinces, Iran. Sequences were analyzed using bioinformatics software.

Results: There were 15 single nucleotide polymorphisms (SNPs) as a transition and transversion mutations. Of 9 mutations in exon I There was a mixture of two bases at positions 1226 (G/C) and 1473 (G/T, C) mismatches lead to amino acid substitutions in V260L, G342A, G342V positions. Of 6 mutations in exon II region, the last one (T/A) was not silent and lead to F407Y substitutions and at least 80% of the samples had F407Y mutation. Genotyping results showed that more than 85% of specimens have at least one of these mutations.

Discussion: The Iranian housefly population was composed of five insensitive and a sensitive allele. The emergence of multiple alleles in field population might due to the application of different pesticides/insecticides during long periods on the Iranian population. Development of an effective control strategy for such populations will be difficult.

Keywords: Housefly, Acetylcholinesterase, Polymorphism, Novel mutation.

Detection of anti-*Toxoplasma gondii* Antibodies in Chronic Myeloid leukemia and Acute Myeloid leukemia Patients

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Abstract

Introduction: Infection of *Toxoplasma gondii* is a worldwide distribution. Toxoplasmosis in patients who are immunocompromised by virtue of underlying leukemia disease has received relatively little attention. This study was aimed to evaluate IgG and IgM antibodies of *T. gondii* and to minimize the role of *T. gondii* and opportunistic infection complication at the early stage of infection in leukemia patients.

Materials and Methods: The purpose of this assay was to measure anti-*T. gondii* IgG and IgM antibodies by enzyme linked immunosorbent assay (ELISA) technique in leukemia patients.

Results: IgG antibodies against *T. gondii* were detected by ELISA in 96 (56.4%) leukemia patients and 72 (42.4%) control group. IgM antibodies were found in 10 patients (5.9%) with leukemia and 3 (1.8%) in the corresponding.

Conclusion: Our finding indicated that leukemia patients under immunosuppressive condition should not be neglected. Toxoplasmosis in leukemia patients as a main risk factor is considered, meanwhile in some patients, due to possibility of the presence of secondary infection that leads to severe toxoplasmosis.

Keywords: acute myeloid leukemia, chronic myeloid leukemia, *Toxoplasma gondii*.

***Demodex* Spp., a major cause of rosacea**

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Objectives: Rosacea is a chronic inflammatory dermatosis, affecting 1% to 10% of the population worldwide. The most seen clinical pictures are telangiectasia, flushing, persistent erythema, papules, and pustules on the center of the face. The present study aimed to evaluate the prevalence of infestation with *Demodex* spp. among patients with rosacea refractory to the common medications.

Materials and methods: In this cross-sectional study, 51 patients with rosacea refractory to common medication referred to Dr. Sharifi Medical Laboratory, Isfahan, Iran, during 2017-2018 were included in the study. Their face skin was investigated for the presence of all stages of *Demodex* spp. with the direct microscopic examination of about 1 cm² sized of affected skin area by squeezing.

Results: Of the 50 patients, 37 (74%) and 13 (26 %) were female and male, respectively. The mean age of patients was 29.5±7.2 (range: 14-45 years old). Considering the infestation, 35 (70%) out of 50 patients had the mite in their facial skin. The mean number of mite/cm² of skin was 3.1±2.7 (range: 1-11). The positivity was different among sexes, 26 (74.3%) and 9 (25.7%) in females and males, respectively, but was not significant ($P=0.602$). On the other hand, the mean number of parasite was significantly higher ($P=0.003$) in females (3.56±3/cm²) compared to males.

Conclusion: Infestation with *Demodex* spp., can be one of the major causes of rosacea which is supporting the previous publications on the field. The very high prevalence of the infestation in this study may be biased by the study population, which was the patients who were desperately seeking the cure of their rosacea, but failed.

Keywords: *Demodex*, rosacea, mite

Infestation with *Demodex* Spp. among patients with hair loss

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Objectives: Hair loss can be very unpleasant, especially among young people. It is caused by a variety of reasons, such as nutritional insufficiency, sex, genetic factors, etc. The present study aimed to evaluate the frequency of infestation with *Demodex* spp. among patients with head hair loss.

Materials and methods: In this cross-sectional study, 32 patients complaining of hair loss who had referred to Dr. Sharifi Medical Laboratory, Isfahan, Iran, during 2017-2018 were included in the study. Their head skin was investigated for the presence of all stages of *Demodex* spp. with the direct microscopic examination of about 1 cm² sized of affected skin area by squeezing.

Results: Of the 32 patients, 25 (78.1%) and 7 (21.9 %) were female and male, respectively. The mean age of patients was 28.2±7.9 (range: 10-50 years old). Considering the infestation, 5 (15.6%) out of 32 patients had the mite in their head skin. The mean number of mite/cm² of skin was 2.2±0.83 (range: 1-3). Among positive cases, 4 (80%) and 1 (20%) were females and males, respectively. Interestingly, 4 (80%) of patients having mite in their head had the mite in their face simultaneously.

Conclusion: According to the results of the present study the prevalence of the infestation with *Demodex* in the head skin is very low and may not be a very important factor regarding head hair loss.

Keywords: *Demodex*, hair loss, mite

***Toxocara canis* Contamination in Stomach of a Stray Dog**

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Background: *Toxocara canis* is one of the most important zoonotic parasites from Ascarididae family. In the evolution of this parasite, the egg containing the infant is the second stage of infectiousness, the survival of infectious eggs, and of course, is important in the epidemic of this parasite. According to the studies, it can be said that if sufficient oxygen and temperature and humidity are available, then *Toxocara canis* Eggs are months and years alive. In Visceral Immigration Syndrome (VLMS), if the egg containing infectious *Toxocara canis* is infected into the human body, the baby migrates to the organs of the body. Therefore, this parasite is an important disease in human and animal health.

Materials and Methods: In the present study, an individual report on a carcass of a three-month-old dog was referred to a veterinary clinic in Karaj, an autopsy was conducted to determine the cause of animal death.

Results: In the digestive system, a parasitic gastroenteritis with the origin of *Toxocara canis* was observed in the stomach and intestines of the animal. In the count, 53 parasites were removed from the stomach. The presence of worms in the stomach was due to the pulmonary migration of larvae and the swallowing of animals by the animal that caused animal obstruction and death.

Conclusion: This study showed that parasitic infectious diseases in stray dogs in this city are very impressive. Therefore, controlling parasitic diseases is important for the health of humans and animals.

Keywords: Stray dogs, zoonotic parasites, Toxocariasis, Karaj

Higher Prevalence of *Blastocystis Hominis* in Healthy Individuals than Patients with Gastrointestinal Symptoms from Ahvaz, Southwestern Iran

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ABSTRACT

Aim & Introduction: *Blastocystis*, a common intestinal protozoan of humans and animals, infected more than 1 billion people around the world. Molecular studies has classified *Blastocystis* in 17 subtypes, of which subtypes 1 to 9 have been reported from humans, in both healthy individuals and patients with gastrointestinal (GI) symptoms. The present study aimed to investigate the subtype variation of *Blastocystis* among patients admitted to the Ahvaz hospitals.

Methods: 345 fecal samples including 151 GI patients and 194 healthy individuals were examined by microscopy, culture and PCR-sequencing techniques to determine *Blastocystis* frequency and subtype(ST) variation.

Results: The occurrence of *Blastocystis* was detected 56 (16.2%) and 85 (24.6%) by microscopy, culture and PCR methods, respectively. Out of the 85 positive patients, 60 (70.6%) were asymptomatic and 25 (29.4%) were symptomatic. The results of 41 successfully sequenced isolates identified 8 (19.5%), 8 (19.5%), and 25 (61.0%) ST1, ST2, and ST3, respectively.

Discussion & Conclusion: In this study, among the 41 sequenced isolates, ST3 (61.0%) was more prevalent than ST1 (19.5%) and ST2 (19.5%). The small size of sequenced samples did not allow us to confirm ST3 as a predominance ST in Ahvaz inhabitants. This study has found that *Blastocystis* was more common in healthy individuals than GI patients.

Seroprevalence and Molecular characterization of *Neospora caninum* from Animal Hosts in Qazvin Province

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Introduction & Objectives: *Neospora caninum* is a coccidian parasite of animals. It is a major pathogen for cattle and dogs and it occasionally causes clinical infections in horses, goats, sheep, and deer. Domestic dogs are the only known definitive hosts for *N. caninum*. Transplacental transmission is considered the major route of transmission of *N. caninum* in cattle. The objective of this study was Seroprevalence and Molecular characterization of *Neospora caninum* from animal hosts in Qazvin province.

Materials and methods: Of 98 blood samples of cattle and calves were collected from industrial dairy farms. Serum samples of cattle and Apparently healthy calves were tested for detecting antibodies against *N. caninum* antigen by using the indirect ELISA test, and detecting DNA of *N. caninum*, using the PCR and nested-pcr.

Results: Of 49 cattle samples 14 (28/57%) and of 49 calves 6 (12/24%) reacted positively to *N. caninum*.

Conclusion: According to the survey, transplacental transmission is the main source of contamination in herds. It is one of the most efficiently transmitted parasite of cattle and up to 90% of cattle in some herds are infected. The rate of transplacental transmission of *N. caninum* infection in Apparently healthy calves were calculated as 42.58%.

Keywords: Seroprevalence, Molecular characterization, *Neospora caninum*

Study of Medical Parasitology M.Sc. Curriculum Contents in view of Lecturers and Students

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Abstract

Background: According to specialist's opinions, it is necessary to revise curriculums every five years after the last revise. The aim of the study was to review of the MSc curriculum of Parasitology for increasing Students ability.

Method: In this study, we requested university lecturers and graduate students to participate in the primary survey and give their opinions concerning the review of curriculum of Master degree of parasitology and need to add or delete any courses. Then, results of 60 questioners that exactly filled by 30 university lecturers and 30 MSc graduates, revised and results were presented based on the several criterions, including compulsory / specificity / importance of the lessons.

Results: the results show that it is necessary two new courses, namely new technologically detecting parasites and taxonomy, to be added to the specialized courses. Furthermore, it was proposed that biotechnology, technical language, basic medical helminthology, applied biostatistics and the use of computer software's, geography of parasitic diseases in Iran, bioinformatics, principles of epidemiology, molecular and cellular biology and teaching methods also to be added as compensatory courses, which are other results of this research in order to improve the curriculum of Master of Parasitology in Iran.

Discussion and conclusion: The results showed that the current approved specific courses are desirable but it is necessary to revise the remedial courses. Our proposed compensatory courses have designed to follow the development of new technologies and the need to update the scientific content.

Keywords: Curriculum, Parasitology, M.Sc.

Anthelmintic Effects of Some Nanoparticles against *Dicrocoelium dendriticum*

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Introduction: Dicrocoeliasis is a considerable parasitic disease in grazing livestock with economic and public health importance caused by the small liver fluke, *Dicrocoelium dendriticum*. Little information is available on the anthelmintic effects of nanoparticles for drug delivery. In the present study, some nanoparticles such as TiO₂/Ag₂O, ZnO/Ag₂O, Ag₂O, AgO, and ZnO/Ag nanoparticles were examined for their possible, in vitro anthelmintic effects against *D. dendriticum*.

Material and Methods: Adult *D. dendriticum* were collected from the liver of freshly slaughtered sheep and goats at the abattoir of Kashan, Iran. The worms were washed and carefully identified, and then four parasites were transported in each 24 plate-well containing RPMI medium and incubated for 72 h at 37 °C in an atmosphere of 5% CO₂ with different concentrations (1, 3 and 6 mg/ml) of the nanoparticles. At the end of each concentration/time, two ml of 0.1% eosin solution was added to each parasite and mixed before being incubated for 30 min. In the viability test, the live *D. dendriticum* is not stained with eosin, while the dead parasites are stained. The parasite mobility and mortality were recorded at different time intervals for 72 h and for each concentration/time, the numbers of live and dead helminths were counted and the mean percentage across the three samples to be calculated. Statistical significances of differences among treatments were determined by use of analysis of variance and covariance (ANOVA), followed by Tukey's pair-wise comparisons. A significant difference was set at a P value <0.05.

Results: The flukes were exposed in triplicate at 1, 3 and 6 mg/ml mg/L to each nanoparticle. The efficacy was assessed as the mortality rate based on the number of live and dead flukes after 12, and 24 h post-exposure. TiO₂/Ag₂O, ZnO/Ag₂O, Ag₂O, AgO, and ZnO/Ag had an in vitro anthelmintic effect. The motility was seriously decreased with the increase in incubation period and concentration of the all nanoparticles. The untreated control worms remained active up to 72 h; however, a slight decrease was observed at the end of incubation period.

Conclusion: It is concluded that five nanoparticles had promising anthelmintic effects against *D. dendriticum*. Further studies on toxicity and in vivo biological evaluation in ruminant models might help to determine the anthelmintic potential of these nanocomposites.

Keywords: *Dicrocoelium dendriticum*, anthelmintic, nanoparticles, mobility mortality

Molecular Analysis of *Aquaglyceroporin 1* Gene in Non-Healing Clinical Isolates Obtained from Patients with Cutaneous Leishmaniasis from Central of Iran

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Abstract

Background: Regarding the antimonial-resistant of *Leishmania* spp., understanding of related mechanism is necessary. One of the most important involved molecules is aquaglyceropin1 (AQP1). The aim of this study was molecular analysis of *AQP1* gene from antimonial-resistant clinical isolates and its expression.

Methods: Overall, 150 patients with cutaneous leishmaniasis referring to the reference laboratories of Yazd and Varzaneh, located 105km southeast of Isfahan and 240 km away from Yazd, were assessed from Jun 2015 to Dec 2017. After sampling, staining was done and evaluated for Leishman by microscope. Samples were collected in RNA later solution for gene expression analysis in non-healing isolates. DNA extraction was performed from each slide with Leishman body. All patients with *L. major* isolates detected by ITS1-PCR-RFLP were followed for finding the resistant isolates, consequence of molecular characterization of *AQP1* using PCR-RFLP. Gene expression of *AQP1* from all resistant isolates was assessed in comparison with the one in a sensitive isolate. Statistical analysis was done using SPSS. The significance level was considered ≤ 0.05 .

Results: Five isolates were detected as antimonial resistant. Molecular detection and identification were appeared that all were *L. major*. The molecular characterization of *AQP1* showed G562A mutation. Gene expression of *AQP1* in resistant isolates showed 1.67 fold higher than the sensitive isolate.

Conclusion: We reported a new point mutation of G562A in *AQP1* gene involved in molecular mechanism in resistant isolates.

Keywords: Leishmaniasis, Cutaneous; Drug resistance, Antimony

Epidemiology and Identification of *Trichomonas vaginalis* Genotypes in women in Southeast of Iran by Actin-Gene PCR-RFLP method

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Abstract: Trichomoniasis is one of the most common sexually transmitted diseases in the world, which is caused by *Trichomonas vaginitis*. As we didn't have any information about the genetic variation, prevalence and related factors that affect the disease, this study was done.

Material and method: Of 500 women patients who were referred to hospitals of Zabol and Zahedan, Twenty-five positive clinical samples from vaginal discharge and urine were isolated by culture during 1959-1969. First DNA extraction was performed and then all samples were subjected to PCR using the nested-PCR method. Six different genotypes of actin gene have been identified by PCR-RFLP in *Trichomonas vaginalis* in Zahedan and Zabol. All of the PCR products were subjected to *HindIII* and *RsaI* and *MesI* enzymes. All participants completed a questionnaire recommended by gynecologists and midwifery experts.

Result: As a result, genotypes, including H, G, E, I, N were obtained in this study. The dominant genotype of *Trichomonas vaginalis* in Zahedan and Zabol is genotype E. There was a significant difference between the type of clinical symptoms and the level of infection ($p=0.0001$).

Conclusion: Control of disease as a health problem must be conducted based epidemiological and genetic method. Control of the disease is related to education and drug resistance or sensitivity related to genetic variation and epidemiological factors.

Keywords: *Trichomonas vaginalis*, Actin gene, Zahedan, Zabol, epidemiology

Prevalence of Sarcocystosis in Slaughtered Carcasses and Histopathology of Them, Southeast of Iran

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Abstract: *Sarcocystis* spp. are common parasites and in terms of economics and pathogenicity in domestic animals is important. The purpose of this work was to define the rate of contamination of slaughtered carcasses of cattle to *Sarcocystis* using digestive and histopathological methods in Southeast Iran.

Material and Method: In this descriptive-laboratory study during one year, 500 carcasses were examined and isolated bradyzoites of *Sarcocystis* with the digest method. Also were considered Tissue samples from the esophagus and diaphragm for pathologic studies and stained with Hematoxylin and Eosin of sections of histopathological. The results showed that the highest contaminations were in imported male animals aged 2 to 3 years old in the spring. There was a significant difference ($P < 0.05$) in the prevalence rate with the sex and racing of cattle, but no significant difference ($P > 0.05$) in the prevalence rate with age and season.

Conclusion: Infection with *Sarcocystis* is common in oxen in this region. The imported cattle are more infected. It seems that racing and the environmental condition affects the prevalence of *Sarcocystosis*.

Keywords: Slaughter, Cattle, Histopathology, *Sarcocystis*

Evaluation of the Prevalence of *Dermanyssus gallinae* in Ornamental Birds as a Potential in Human Contamination

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Abstract: *Dermanyssus gallinae* is an ectoparasite of poultry and has been played a main role as a vector of several major pathogens. It has a wide range of hosts including several species of wild and ornamental birds and mammals include humans.

Procedure: In order to investigate the prevalence of *Dermanyssus gallinae* in ornamental birds, seven sale centers of ornamental birds were visited in Shahrekord town and a total of 40 cages of birds were randomly selected and checked. Samples were transferred to the laboratory of parasitology of Shahrekord University and were separated and mounted. Then were diagnosed using diagnosis keys of the mites. A total number of about 2,000 mites were found from the specimens.

Results: The result showed that all cages and birds were infested with *Dermanyssus gallinae* (Red Mite).

Discussion and conclusion: Due to the ability of transmission of such parasite to humans and also since ornamental birds are kept at homes, it is possible to have problems via human biting and possible disease transmissions. So care should be taken to protect humans against this parasite biting and possible diseases which are transmitted.

Keywords: *Dermanyssus*, biting mites, public health.

Connection Between Human Cercarial Dermatitis (HCD) in Mazandaran Province (North of Iran) and the Presence of Nasal Bird Schistosomes in Final and Intermediate Hosts

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Abstract

Background: Avian schistosomes are considered as main causative agents of human cercarial dermatitis (HCD) in Iran. The study was conducted to determine bird schistosomes in their final and intermediate hosts, in main wetlands of Mazandaran.

Methods: A total of 255 domestic and wild ducks were collected and the infection of nasal tissues of five (*Anas platyrhynchos domesticus*, *Aythya ferina*, *Cairina moschata*, *Anas platyrhynchos* and *Spatula clypeata*) species were analyzed using morphological techniques. Also, 1,687 freshwater snails were collected and surveyed by cercarial shedding and crushing tests. Detection of HCD was performed for the presence of clinical symptoms of itching and maculopapular rashes by physical examination.

Results: Of 255 ducks, in 41 (16%) infection with nasal *Trichobilharzia* spp. were recognized by observing eggs and/or adult worms. The most infected ducks were *Anas clypeata* and *Anas platyrhynchos domesticus*. Overall, 0.17% of snails were infected with avian schistosomes. Also, clinical examination of 951 farmers revealed that 588 (61.82%) of them were suffered from HCD.

Conclusions: Our data suggest that domestic ducks could play a prominent role as a reservoir host for establishing life cycle of *Trichobilharzia* in the area. Also, existence of domestic reservoir ducks and suitable snail hosts in ponds and paddy fields of this area, climate conditions of the wet lands in Mazandaran leads to a high incidence of HCD.

Keywords: Bird schistosomes, final hosts, intermediate hosts, cercarial dermatitis, Mazandaran Province

Study on Helminth Parasites of Wild Canines of Moghan plain, Ardabil Province, Northwest Iran with Special Reference to Zoonotic Species

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Abstract

Introduction and Objectives: Canines are one of the most important sources of zoonotic infections in a wide range of countries throughout the world, like in Iran. This study aimed to determine Helminth parasites of wild canines of Moghan plain, Ardabil Province, northwest Iran, with special reference to zoonotic species.

Methods: During 2017-2018 the carcasses of 60 wild canines from road accidents, including 25 jackals, 15 foxes, and 20 wild dogs were collected from different locations of Moghan plain and after necropsy different tissues examined for infectivity with helminthic parasites.

Results: Overall, 49 canines (81.7%) were infected with at least with one helminthic parasite. No trematode was found. The species and percent of infections were as follows:

-Cestoda: *Mesocestoides lineatus* 38.3%; *Taenia hydatigena* 16.7%; *Echinococcus granulosus* 8.3%; *Dipylidium caninum* 6.7%; *Joyxiella echinorhyncoides* 1.7%.

-Nematoda: *Toxascaris leonina* 18.3%; *Rictularia sp.* 11.7%; *Toxocara canis* 11.7%; *Trichinella sp.* 3.3%; *Dirofilaria immitis* 3.3%; *Physaloptera praeputialis* 1.7%.

-Acanthocephala: *Macracanthorhynchus hirudinaceus* 10%.

Discussion and conclusion: Among the 12 species identified, 9 species were zoonotic. Most of them have public health importance and *Echinococcus granulosus* are the most important zoonotic species.

Keywords: Helminth, wild Canine, Zoonose, Iran

Prevalence of Hydatidosis and Sarcocystosis in Slaughtered Livestock in Abattoir of Golestan Province, Northern Iran, 2017–2019

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Abstract

Introduction and objectives: Hydatidosis is a zoonosis disease caused by larval stage of the genus *Echinococcus*, a tiny tapeworm. The definitive hosts are carnivorous predators such as dogs and wolves. Cattle, sheep, goats, camels and wild herbivores serve as the usual intermediate hosts harboring the hydatid cyst via the ingestion of helminths' eggs. *Sarcocystosis* is one of the important protozoan parasite in phylum Apicomplexa causing the *sarcocystosis* which is a zoonotic disease. Both hydatidosis and sarcocystosis are responsible for economic losses to ranchers. This retrospective study is a survey on prevalence of hydatidosis and sarcocystosis in slaughtered animal, in Golestan province.

Materials and Methods: The information of a three years' period from April 2017 to March 2019 obtained from Veterinary Directorate of Golestan province and analyzed considering to different hosts.

Results: A total of 720804 slaughtered livestock were examined through visual inspection including 41922 cattle, 621261 sheep and 57621 goats. Overall, 36443 (5.05%) were infected by hydatid cyst and 196 (0.02%) to *Sarcocystis* spp. Approximately one percent of cattle, 5.74% of sheep and 0.6% of goats were found infected by hydatid cyst. Roughly 0.5% of sheep was infected to *Sarcocystis* spp. Interestingly, cattle and goats were not contaminated by *Sarcocystis* spp.

Conclusions: Due to considerable prevalence of hydatidosis and sarcocystosis in sheep, it is appeared that sheep is the most important host for these parasitic diseases in this area. So, prevention and control strategies should be executed on livestock rising.

Keywords: Hydatidosis, *Echinococcus* spp, *Sarcocystosis*, Livestock, Golestan.

In Vitro and in Vivo Survey of Ethyl Acetate Extract of *Acoruscalamus* Rhizome on *Toxoplasma gondii*

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Background & Aim: Toxoplasmosis is a zoonosis disease that can cause a variety range of manifestations in human specially fetus duration and immunodeficiency conditions. Due to toxicity and side effects of current, we evaluated in vivo and in vitro effects of ethyl acetate extract of *Acoruscalamus* root on *Toxoplasma gondii*.

Methods & Materials: The plant rhizomes were collected from Sari, North of Iran. Ethyl acetate extract was collected using soxhlet apparatus. The mortality effect of different concentration (1, 2, 4, 8, 16, 32, 64, 128 and 256 µg/ml) of the extract on tachyzoites was assessed by flowcytometry and Propidium Iodide. For the assessment of therapy effect, tachyzoites were inoculated intraperitoneally to mice and then different groups administered different concentrations due to oral and intraperitoneal admins.

Results: The plant extract was rich of phenolic compounds (41.27±0.21mg/g) whereas it contains less amount of flavonoids (4.79± 0.01mg/g). Results of in vitro experiments showed there is an inverse relationship between the concentrations and the mortality of the parasites. IC₅₀ of the extract on the parasite was calculated 200.01±7.74 (µg/ml). A significant difference was seen at maximum concentration (256µg/ml) with gavage administration in longevity of the infected mice (P<0.031).

Discussion & Conclusion: Our study demonstrated that *Acoruscalamus* extract had significant activities against *T.gondii* in vivo and invitro which is connected to high amount of phenolic compounds. We suggest the effects of fractions on the parasite. Alternatively, the administrations types and dosage of the extract on the parasite must be evaluated.

Keywords: *Toxoplasma gondii*, *Acoruscalamus*, in vivo and in vitro

Serological and Molecular Survey of *Toxoplasma gondii* Infection in Hemodialysis Patients with Chronic Renal Disease in Zahedan, Iran

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ABSTRACT

Toxoplasma gondii is one of the most prevalent zoonotic opportunistic parasitic infections throughout the world. This study was conducted to examine toxoplasmosis in 119 hemodialysis patients (HP) and a control group in Zahedan City, southeastern Iran, in 2017. The molecular and serological detection of toxoplasmosis was conducted using nested-PCR and ELISA in all participants.

IgM and IgG seropositivity was diagnosed in individuals experiencing hemodialysis (44.5% and 0%, respectively), while these values were significantly different from the controls (23.5% and 0.8%, respectively). *T. gondii* DNA was identified in 35/119 individuals (29.4%) only in hemodialysis patients.

In conclusion, preliminary *T. gondii* infection screening is required using serological techniques, particularly in hemodialysis patients (HP) who are frequently exposed to hemodialysis so as to stop infection dissemination.

Malaria Diagnostic Test: Compare Multiplex/Nested-PCR, LAMP and Light Microscopy with Nested-PCR in South-Eastern Iran: A Country Close to Malaria Elimination

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Abstract

Topic: Malaria is one of the most serious health problems in different countries, including southeastern Iran. Therefore, Accurate diagnosis of malaria is important because Iran is at the stage of eliminating the disease.

Materials and Methods: A cross-sectional study was performed on 105 who were suspected to be positive for Malaria infection in Sistan and Baluchistan, Iran. Blood smears (thin and thick films) were stained with 10% Giemsa. Blood smears were examined by two experienced microscopists blinded to each other's results. DNA was extracted from the prepared thin and thick films for molecular methods. Results of Multiplex/nested PCR, LAMP method and Light Microscopy (LM) compare with Nested PCR, and analyzed with using SPSS 16 software package.

Results: of 105 case, LM, 52(49.5%), nPCR and multiplex/nested PCR 58(55.2%) and LAMP method 63(60%) were positive for malaria. The sensitivity (Sn), specificity (Sp) and Kc were 92.06%, 100%, and 0.903 for LAMP and 100%, 100%, and 1, for Multiplex/nested PCR, respectively. eight cases of co-infection (*Plasmodium vivax* and *falciparum*) that not detected by the LM method were diagnosed by multiplex/nested PCR and LAMP methods.

Conclusion: The use of LAMP is a good solution in co-infection cases. In addition, the multiplex/nested PCR can be a good alternative to nPCR by reducing the testing time and cost.

Keywords: Malaria, multiplex/nested PCR, LAMP, Light Microscopy, Iran

High Resolution Melting Analysis as an Accurate Method for Identifying *Leishmania infantum* Canine Serum Samples

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ABSTRACT

Background & objectives: *Leishmania (L.) infantum* is the principal agent of visceral leishmaniasis (VL) in the Mediterranean and American regions. So far different molecular methods including high resolution melting (HRM) analysis have been developed for detecting and identifying *L. infantum* infection. HRM assay is an automated molecular method which detects and identifies different genus and species of infectious agents. This study aimed to diagnose and identify *Leishmania* infection caused by *L. infantum* species using real-time PCR coupled with HRM assay in the serum samples in comparison with anti-*L. infantum* antibodies obtained using direct agglutination test (DAT), in domestic and wild canines of northeastern Iran.

Methods: Serum samples of 15 foxes, 14 jackals, seven domestic dogs and three wolves were collected in some villages around Shirvan and Bojnourd districts from the northeast regions of Iran during 2014–15. Initially, all the collected serum samples were tested by DAT for the detection of anti-*L. infantum* antibodies. Afterwards, genomic DNA was extracted from the samples and tested by real-time PCR–HRM analysis targeting hsp70, ITS1 and gp63 genes. The level of agreement between DAT and HRM assay were analysed statistically.

Results: Out of the 39 serum samples, eight showed anti-*L. infantum* antibodies at titre 1: 80 while only one of them showed anti-*L. infantum* antibodies at titre 1 : 160. All the nine seropositive samples showed positive results with HRM analysis. Additionally, three DAT negative serum samples were also found positive in the HRM technique. Altogether, 12 out of the 39 DNA samples showed positive results in HRM analysis. Among the three gene sequences

used, gp63 was best for separation and identification of species.

Interpretation & conclusion: HRM analysis targeting hsp70, ITS1 and gp63 genes can be used as a highly sensitive technique for the screening and early detection of *L. infantum* infection in the wild and domestic canines. It has higher accuracy than DAT and allows detection and discrimination of different *Leishmania* species responsible for the *Leishmaniasis*.

Keywords: gp63; high resolution melting; hsp70; ITS1; *Leishmania infantum*; visceral leishmaniasis

Efficacy and Safety *Curcuma zadoaria* L. to inactivate the hydatid cyst protoscoleces

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Background: Today, available chemical drugs have shown severe complications during hydatid cyst surgery. Here we evaluated the chemical composition of *Curcuma zadoaria* essential oil and investigate its efficacy and safety against hydatid cyst protoscoleces.

Methods: Collected protoscoleces from liver fertile hydatid cysts of infected sheep were exposed to the different concentrations of the essential oil (75, 150, 300 µl/mL) for 5-30 min in vitro and ex vivo. Then viability of protoscoleces was evaluated using the eosin exclusion test. In next step, 24 male NMRI mice were applied to assess the toxicity of *C. zadoaria* essential oil by measuring biochemical and hematological parameters.

Results: *C. zadoaria* essential oil had a powerful protoscolicidal activity in vitro so that at the 300 and 150 µl/ml entirely eliminates the parasite after 5 and 10 minutes; whereas at lower doses demonstrated weak protoscolicidal activity. Ex vivo assay, requiring more time to show a potent protoscolicidal activity. *C. zadoaria* essential oil at the concentrations of 300 and 150 µl/mL after exposure time of 7 and 12 min, killed 100% of protoscoleces within the hydatid cyst, respectively. After intra-peritoneal injection of the *C. zadoaria* essential oil for 2 weeks, no significant difference ($p > 0.05$) was observed in the clinical chemistry and hematologic parameters at the doses 0.15, 0.3, 0.6 mL/kg.

Conclusion: The obtained results exhibited that *C. zadoaria* essential oil had the favorable protoscolicidal activity on hydatid cyst protoscoleces. However, more and supplementary works are required to verify these findings through assessing in clinical subjects.

Keywords: GC/MS, cystic echinococcosis, *Echinococcus granulosus*, protoscoleces

A Survey on *Toxoplasma gondii* Infection in Aborted Fetuses of Sheep Using PCR in North Khorasan Province, Iran

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Abstract

Introduction: *Toxoplasma gondii* is a zoonotic obligate intracellular protozoan parasite that infects warm-blooded animals as well as humans worldwide. The purpose of this study was to delineate the prevalence of *Toxoplasma* infection in aborted fetuses of sheep in the North Khorasan province, Iran.

Material & Method: Three hundred and ninety-nine samples of liver (133 samples), placenta (133 samples), and brain (133 samples) from 133 aborted fetuses of sheep were collected from 2015 to 2017. The ages of aborted fetuses were higher than 120 days in this study.

Results: According to the samples, sixteen out of 133 aborted fetuses of sheep were infected with *T.gondii*. *Toxoplasma* DNA was found in placenta (68.75%) and liver (31.25%) samples of infected fetuses using PCR method. The highest and lowest rates of *Toxoplasma* infection were observed during 2016 and 2017, respectively. Shirvan and Faruj provinces were recognized as the two most infected districts among others. There was a significant difference between year and abortion rate in sheep due to infection by *Toxoplasma* parasite ($P < 0.05$). Furthermore, no significant difference between the prevalence of *T.gondii* infection and aborted fetuses was seen ($P > 0.05$) in different areas.

Conclusion: According to the present, *T.gondii* infection can be one of the causes of fetus abortion of sheep in the North Khorasan province, Iran.

Keywords: Sheep abortion; *Toxoplasma gondii*; PCR, Iran

Seroepidemiological Survey of Toxoplasmosis among Pregnant and Abortive Women of Gonabad City

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Introduction: Maternal immunity to *Toxoplasma gondii* is very important during pregnancy. Non-immunized women may be at risk of toxoplasmosis during pregnancy. This parasite is able to pass through the placenta to the fetus and causes severe complications in the fetus. The aim of this study is to investigate the seroepidemiology of *Toxoplasma* infection in pregnant women of Gonabad.

Materials and Methods: Three hundred blood samples were collected from pregnant women and abortive Women of 18-40 years old referred to Gonabad 'shealth centers and hospitals. The IgG and IgM antibody titers were measured by ELISA method.

Results: Samples were taken from 252 (84.0%) pregnant women and 48 (16.0%) women with abortion. The average age of these women was 29.23 ± 6.24 years. Among these subjects, 56 (22.2%) of pregnant women and 15 (31.3%) of women with abortion history had anti-*Toxoplasma* IgG antibodies while 196 (77.8%) of pregnant women and 33 (68.7%) of women with abortion history did not have this specific antibody. Also, 3 (1.2%) pregnant women had IgM antibodies while this antibody was not seen in any women with a history of abortion. In this study, the prevalence of toxoplasmosis was 23.6%.

Conclusion: According to the results, 76.33% of pregnant and abortive women in Gonabad city have no history of *Toxoplasma* infection. Therefore, they are prone to toxoplasmosis infection during their pregnancies. In this regard, it is necessary to establish public health and preventive actions as well as rapid diagnosis to eliminate risk factors during the pregnancy.

Keywords: Seroepidemiology, *Toxoplasma* infection, Abortive women, Pregnant women,

The Seroepidemiology of Hydatid Cyst Disease in At-Risk Individuals in Gonabad City of Khorasan Razavi

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Introduction: Hydatid cyst is one of the most common diseases of humans and livestock that causes many economic and health damages in the world every year and then, it is necessary to know its health and economics aspect. It is also considered to be the first parasitic worm in the world. The purpose of this study was to determine the seroepidemiology of human hydatid cyst in people at risk in the city of Gonabad in Khorasan Razavi province.

Materials and Method: The study was a cross-sectional and experimental study. 369 samples of at risk with hydatid cyst disease were taken from 11 villages and 2 cities of Khorasan Razavi. Then, the samples were tested by ELISA method and for testing the samples we used the market-leading commercial kits from PishtazTeb Company. Absorption (OD) of negative control samples at a wavelength of 450 nm was considered as cut off point. The results were analyzed by SPSS software version-22.

Results: From 369 people at risk, with hydatid cyst disease, numbers of women and men were 133 and 235, respectively. Of the total samples, 36 (9.8%) samples were positive with ELISA and there was a significant correlation between the sex with hydatid cyst, as the prevalence was higher in men (P value=0/003), and there was a significant difference between the number of livestock and the hydatid cyst disease (P value=0/0001).

Conclusion: In this study, 9.8% of the people at risk were infected with hydatid cyst disease, who were not aware of the disease themselves, and this shows the high level of the prevalence of hydatid cyst in Gonabad city. Then, the ELISA test can be used as a screening test in the areas where animal husbandry is considered as the main occupation.

Keywords: Hydatid cyst, Seroepidemiology, ELISA, Gonabad

Frequency of Intestinal Parasites in HIV-Positive Patients, Bandar Abbas, Iran, 2018

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Introduction and Objectives: Intestinal parasites are one of the most important causes of infectious diseases in the individuals with immunodeficiency syndrome. Transmission of fecal-oral infections occurs through direct and indirect contact with food and contaminated drinks. Intestinal parasites, including opportunistic parasites in HIV-positive patients, may cause severe digestive disorders and even death. Due to the importance of infectious diseases in these patients, the presence of these parasites was investigated.

Materials and Methods: In this descriptive cross-sectional study, we studied the fecal specimen of HIV positive patients under the care of the center for Behavioral Disease Counseling (BDC) of Bandar Abbas. The diagnosis was made on the basis of the direct wet mount (normal saline and lugol), formalin–ether concentration method, and Ziehl–Neelsen and trichromestained slides.

Results: Out of the 133 patients, 80 were males (60.25) and 53 were females (39.8%). The mean age of the patients was 42.15 year, of which the youngest were 12 and the oldest 82. There were no clinical signs of intestinal parasites, including diarrhea, in any of the patients. The frequency of intestinal parasites was 2.4% (3 cases), including *Giardia lamblia*, *Hymenolepis nana* and the other one simultaneously infected with *Entamoeba coli* and *Blastosistis hominis*. None of the patients had intestinal coccidial parasites.

Conclusion: Evaluating and monitoring the therapeutic process of the patients in BDC lead to less intestinal parasites.

Keywords: Intestinal parasites, AIDS, Bandar Abbas, Iran

A Comparative Evaluation Between Anti - Ascariasis Effect of Aloe Vera, Allium Sativum and Piperazin in Native Dogs

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Abstract:

Introduction and aims: Piperazin applied in veterinary medicine for ascariasis but it has side effects such as stomach pain and diarrhea, so our aim is to replace Aloe Vera and garlic as herbal medicines of Iran to kill ascariasis parasite in the GI of dogs.

Research method: In recent study, 20 native dog with mean weight 30kg selected in 4 groups, randomly from 2 sex. First group (control) took 0.5cc/kg piperazin daily as orally syrup for 2 days and this program repeated as same in 21 day later. Second group (treatment) received the Aloe Vera gel orally as 5mg/kg, orally, one time in the day for 2 days and for 2 days after 21 day. Third group (treatment) received Garlic autoclaved powder as 5mg/kg in 2 days daily, orally and for 2 days after 3 week. Forth group received all of three above drug for that period time.

Findings: The dogs of first and forth groups had: Excessive diarrhea as piperazin side effect and no GI ascariasis. But second and third groups had no side effects and no GI ascariasis, two.

Discussion and Conclusion: In current report, both Aloe Vera and garlic as herbal medicine had no side effects in comparison to piperazin so we suggested replace them in dogs as herbal anti- ascariasis drug because is safe and potent and available.

Keywords: Piperazin, Aloe Vera, Allium Sativum, anti-ascariasis, dog.

A Comparative Evaluation between Antiparasite Effect of Mint and Ivermectin in Native Dogs of Tehran Province

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Abstract:

Introduction and aims: Ivermectin is a commonly used as antiparasite drug in veterinary medicine but there are many reports related to ivermectin toxicities so our aim is to replace mint as herbal medicine of Iran to kill GI parasites in dogs.

Research method: In recent study, 9 native dog with mean weight 25kg selected in 3 groups, randomly. These dogs are from 2 sex. First group (control) injected by 2mg/kg Ivermectin daily for 3 days. Second group (treatment) received the mint autoclaved powder as 5mg/kg, orally, one time in the day for 3 days. Third group (treatment) received both of them in 3 days.

Findings: The dogs of first and third groups had: Excessive drooling, ataxia, tremor, dyspnea and anorexia as ivermectin side effects and no parasite. CBC did not help to us. But second group had no side effects and no GI parasite, two.

Discussion and Conclusion: In current report, it seems that mint as herbal medicine had no side effects in comparison to Ivermectin so we suggested replace it in dogs as herbal antiparasite drug because is safe and potent and available.

Keywords: Ivermectin, mint, antiparasite, dog.

A Comparative Evaluation between Antiparasite Effect of Artemisia, Peppermint and Mebendazole in Cats

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Abstract:

Introduction and aims: Mebendazole applied in veterinary medicine to kill parasite but it has interaction with other drugs such as metronidazole and side effects such as stomach pain and diarrhea, so our aim is to replace Artemisia and peppermint as herbal medicines of Iran to kill parasite in the GI of cats.

Research method: In recent study, 12 DSH cat with mean weight 4kg selected in 4 groups, randomly from 2 sex. First group (control) took 5mg/kg mebendazole daily as orally for 5 days. Second group (treatment) received the Artemisia autoclaved powder orally as 5mg/kg one time in the day for 5 days. Third group (treatment) received peppermint autoclaved powder as 3mg/kg in 5 days daily, orally. Forth group received all of three above drug for that period time.

Findings: The cats of first and forth groups had: Excessive diarrhea as mebendazole side effect and no GI ascariasis. But second and third groups had no side effects and no GI ascariasis, two.

Discussion and Conclusion: In current report, both Artemisia and peppermint as herbal medicine had no side effects in comparison to mebendazole so we suggested replace them in cats as herbal antiparasite drug because is safe and potent and available.

Keywords: Mebendazole, Artemisia, Peppermint, antiparasite, cat.

A Comparative Evaluation between Antiparasite Effect of Comomile, Peppermint and Mebendazole in Dogs

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Abstract:

Introduction and aims: Mebendazole applied in veterinary medicine to kill parasite but it has side effects such as stomach pain and diarrhea, so our aim is to replace comomile and peppermint as herbal medicines of Iran to kill parasite in the GI of dogs.

Research method: In recent study, 15 native dog with mean weight 15kg selected in 4 groups, randomly from 2 sex. First group (control) took 5mg/kg mebendazole daily as orally for 5 days. Second group (treatment) received the comomile autoclaved powder orally as 5mg/kg one time in the day for 5 days. Third group (treatment) received peppermint autoclaved powder as 3mg/kg in 5 days daily, orally. Forth group received all of three above drug for that period time.

Findings: The dogs of first and forth groups had: Excessive diarrhea as mebendazole side effect and no GI ascariasis. But second and third groups had no side effects and no GI ascariasis, two.

Discussion and Conclusion: In current report, both Comomile and peppermint as herbal medicine had no side effects in comparison to mebendazole so we suggested replace them in dogs as herbal antiparasite drug because is safe and potent and available.

Keywords: Mebendazole, Comomile, Peppermint, antiparasite, dog.

A Comparative Evaluation between Antiparasite Effect of Tea Tree Oil, Peppermint and Fenbendazole in Cats

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Abstract:

Introduction and aims: fenbendazole applied in veterinary medicine to kill parasite but it has interaction with other drugs such as metronidazole and side effects such as stomach pain and diarrhea, so our aim is to replace Tea tree oil and peppermint as herbal medicines of Iran to kill parasite in the GI of cats.

Research method: In recent study, 20 DSH cat with mean weight 3.5kg selected in 4 groups, randomly from 2 sex. First group (control) took 5mg/kg fenbendazole daily as orally for 5 days. Second group (treatment) received the tea tree oil orally as 3mg/kg one time in the day for 5 days. Third group (treatment) received peppermint autoclaved powder as 3mg/kg in 5 days daily, orally. Forth group received all of three above drug for that period time.

Findings: The cats of first and forth groups had: Excessive diarrhea as fenbendazole side effect and no GI ascariasis. But second and third groups had no side effects and no GI ascariasis, two.

Discussion and Conclusion: In current report, both tea tree oil and peppermint as herbal medicine had no side effects in comparison to fenbendazole so we suggested replace them in cats as herbal antiparasite drug because is safe and potent and available.

Keywords: Fenbendazole, Tea tree oil, Peppermint, antiparasite, cat.

A Comparative Evaluation between Antiparasite Effect of Comomile, Allium Sativum and Piperazin in Dogs

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Abstract:

Introduction and aims: Piperazin is a commonly used as antiparasite drug in veterinary medicine for ascariasis but this drug is expensive and rare in our recent station in Iran and has side effects such as stomach pain and diarrhea, so our aim is to replace Comomile and Allium Sativum (garlic) as herbal medicines of Iran to kill ascariasis parasite in the GI of dogs.

Research method: In recent study, 12 native dog with mean weight 20kg selected in 3 groups, randomly from 2 sex. First group (control) took 0.5cc/kg piperazin daily as orally syrup for 2 days and this program repeated as same in 21 day later. Second group (treatment) received the comomile autoclaved powder orally, 5mg/kg one time in the day for 5 days. Third group (treatment) received Allium Sativum autoclaved powder as 5mg/kg in 5 days daily, orally.

Findings: The dogs of first group had: Excessive diarrhea as piperazin side effect and no GI ascariasis. But second and third groups had no side effects and no GI ascariasis, two.

Discussion and Conclusion: In current report, it seems that comomile and garlic as herbal medicines had no side effects in comparison to piperazin so we suggested replace it in dogs as herbal anti-ascariasis drug because is safe and potent and available.

Keywords: Piperazin, Comomile, Allium Sativum, anti-ascariasis, dog.

Application of Geographical Information System (GIS) in Parasitology

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Abstract

Introduction and objectives: The Geographical Information System (GIS) is a Computer-supported system composed of hardware, software, data, and trained users that communicates between geographical features and various information. In parasitology, GIS offer powerful means for disease mapping, ecological analysis and epidemiological surveillance and have become indispensable tool for processing, analyzing and visualizing spatial data.

Material and methods: In this review, we summarize general aspects of GIS and emphasize the most important applications of these tools in parasitology, including recent advances in territorial sampling, disease mapping, spatial statistics, Bayesian inference, ecological analyses and epidemiological surveillance.

Results: The published articles are mainly in three groups. The first category has examined the incidence or prevalence of parasitic diseases. The second group studies the association of environmental factors, such as climatic parameters with an outbreak of the parasitic disease, which is mainly due to vector born disease, such as Malaria, leishmaniasis and fascioliasis. In the last category, changes in parasitic diseases have been investigated over the years and the outbreak of diseases in the future has been predicted due to various global climatic changes.

Discussion and conclusion: In the past decades, the use of GIS in various sciences, including medical and veterinary parasitology, has spread far too much. Therefore, many projects defined with great grants, have been set up at global organizations to examine and control diseases such as schistosomiasis, malaria, and leishmaniasis by using GIS. GIS training for parasitologists seems to help control parasitic infections.

Keywords: GIS, Parasitology, Spatial analysis, Spatial statistics

A comparative evaluation between anticheytiella effect of Tea tree oil and Permethrin in rabbits

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Abstract:

Introduction and aims: Permethrin is a commonly used as septicide in veterinary medicine but there are many reports related to immunotoxicity so our aim is to replace tea tree oil as herbal medicine to kill cheytiella as external parasite in rabbits.

Research method: In recent study, 15 rabbit with mean weight 2kg selected in 3 groups, randomly. These rabbits are from 2 sex and their skin had this mite. First group (control) sprayed their skin by permethrin powder until 3 days with dose 1 in 1000 mg in one litre water. Second group (treatment) sprayed their skin by tea tree oil with dose 3mg/kg for 3 days. Third group (treatment) received both of them in 3 days.

Findings: The rabbits of whole 3 groups had no mite after 3 days but groups of 1 and 3 had skin rush on their skin but there was not any skin rush on skin of second group rabbits.

Discussion and Conclusion: In current report, it seems that tea tree oil as herbal medicine had no side effects in comparison to permethrin so we suggested replace it in rabbits as herbal antimitte spray.

Keywords: Tea tree oil, Permethrin, antiparasite, rabbit.

Efficacy and safety of *Zataria multiflora* Boiss essential oil against acute toxoplasmosis in mice

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Abstract

Background: Broad spectrums of pharmacological properties, including antimicrobial activity have been attributed to *Zataria multiflora* Boiss (Lamiaceae). The in vivo efficacy and safety of *Z. multiflora* essential oil (ZM-EO) were evaluated against acute toxoplasmosis caused by *Toxoplasma gondii* (Sarcocystidae) in mice.

Methods: Male NMRI mice were orally treated with normal saline (control group) and ZM-EO at the doses of 0.2 and 0.4 mL/kg once a day for 14 days (8 mice in each group). On the 15th day, the mice were infected with 10⁴ tachyzoites of *T. gondii* RH strain by intraperitoneal route. The mortality rate and parasite load were determined in the infected mice. Additionally, 24 mice were applied to examine the sub-acute toxicity of ZM-EO at the above doses after treatment during 14 days.

Results: GC/MS analysis displayed that the key constituents were thymol (45.4%), carvacrol (23%) and p-cymene (10.6%), respectively. Results showed that 100% mortality was observed on the 8th and 9th days in treated mice with the concentrations of 0.2 and 0.4 mL/kg, respectively. The mean number of tachyzoites in the mice treated with 0.2 and 0.4 mL/kg of ZM-EO were 189×10^4 and 76×10^4 cell/mL, respectively, which were meaningfully ($p < 0.05$) reduced compared with the control mice. Results also demonstrated that ZM-EO had no important toxicity on mice.

Conclusion: The results demonstrated the efficacy of ZM-EO against acute toxoplasmosis. Nevertheless, supplementary surveys are mandatory to examine its precise effects, mainly immunomodulatory effect on toxoplasmosis.

Keywords: Prophylactic; essential oil; *Toxoplasma gondii*; treatment; herbal medicines; toxicity

Toxoplasmosis in Alzheimer patients: A systematic review and meta-analysis

Zahra Hosseini Nejad

Introduction: Toxoplasmosis is a major public health concern due to the nature neurotropic and role in the development

of mental and behavioral disorders. Alzheimer disease (AD) is an important nervous disease that results in reduction in the amount of β -amyloid plaque deposition and irreversible loss of neurons in brain. Although a few articles evaluated the association between AD and toxoplasmosis, in the current study we conducted a systematic review and meta-analysis of published studies to investigate the possible association between *Toxoplasma gondii* (*T. gondii*) and AD.

Method: A systematic literature search was conducted using seven electronic databases from inception to 25th of November 2018, with no restriction of language, which looked at toxoplasmosis (as exposure) and AD (as disease). The random effect model was used to determine the odds ratio (OR) using forest plot with 95% confidence interval (CI), and statistical significance was set at 5.0%.

Results: Generally, eight studies containing 3239 subjects (360 patients and 2879 controls) met the eligibility criteria. Then, eight articles were used for meta-analysis with respect to the inclusion and exclusion criteria. The results of the meta-analysis were indicated a common OR of 1.53 (95% CI 1.07-2.18) by random effect model.

Discussion and conclusion: This systematic review and meta-analysis is the first study to demonstrate the relationship between AD and *T. gondii* infection. Based on the results of this study, *T. gondii* can be considered as a risk factor for the development of AD and exacerbation of symptoms, which needs special attention of specialist doctors and patients attendance.

Keywords: *Toxoplasma*, Toxoplasmosis, Prevalence, Seroprevalence, Alzheimer

The evaluation of Toxoplasmosis in the patients with granulomatous lymphadenitis by using PCR in Shiraz during 2009-2014

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Background: Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii* in humans and animals. This disease leads to several clinical signs such as lymphadenopathy, encephalitis, myocarditis and pneumonitis. One type of lymphadenopathy that is mainly appears in the cervical lymph node is granulomatous lymphadenitis. Definite diagnosis of toxoplasmosis in suspected patients is based on PCR. Given the high prevalence of *toxoplasma* in Iran, early diagnosis can reduce the mortality rate and enhance the treatment of the disease. Therefore the aim of present study was the evaluation of toxoplasmosis in the patients with granulomatous lymphadenitis by using PCR.

Material and methods: A total of 84 patients with granulomatous lymphadenitis were evaluated using fine needle aspiration and microscopic methods. The initial diagnosis of all 84 patients was granulomatous lymphadenitis. The PCR method and then electrophoresis in the gel was performed to confirm the diagnosis of toxoplasmosis.

Results: The result of present study showed that PCR enable to identification of 14.2% (12 of 84) of the negative cytology samples that included strains of *T.gondii*.

Conclusion: Considering the high prevalence of *toxoplasma* in Iran and this point that have a lot of mutual symptoms with granulomatous lymphadenitis; it is suggest that people with granulomatous lymphadenitis signs are tested by PCR method for Toxoplasmosis.

Keywords: *Toxoplasma gondii*, Granulomatous lymphadenitis, DNA extraction, PCR.

Evaluation the effect of Myto fade plus on dermanyssidae infestation in some poultry farms in alborz province

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Background: Dermanyssus mites are the most important parasitic problem of laying hens and mothers which causing heavy economic losses in these aviculture. These mites lead to a sharp drop in production by blood thirsting and causing severe stress in hens, while also transmitting other infectious pathogens, including viruses.

Materials and methods: In this study, a non-chemical supplement containing various herbs and some of the consumables as an anti-mite agent have been used and their effects have been evaluated on the reduction of mites population in an aviculture. Subsequently, consuming doses of 0.5, 1, 1.5 kg /T of Mite fade plus (MF+) supplement mixed in seed for 8 weeks, the population of Dermanyssus mites was measured by trapping with specific traps before and during 8 consecutive weeks.

Results: The results showed a significant decrease in the number of mites in all three treatment groups. The number of mites in the groups (MF+ 0.5 kg / t), (MF+ 1 kg / t) and (MF+ 1.5 kg / t) decreased from 54.8 to 17.6, from 50.4 to 13.2 and from 57.3 to 8.5, respectively. The therapeutic effect in these three groups was 8.67%, 79.75% and 85/17%, respectively.

Conclusion: Researchers have greatly focused on the use of non-chemical compounds and various herbs due to the issue of drug resistance, the serious problem of poisoning in poultry meat and produced eggs and complications due to their consumption in human beings. Due to the decrease in the number of mites and the therapeutic effects, the MF+ supplement is an acceptable anti-mite, especially at 1.5 kg/t dose, and is used as a non-chemical anti-mite agent.

Keywords: Myto fade plus, dermanyssidae infestation, poultry farms, alborz province.

Incidence of *Gongylonema* in ruminants slaughtered in Hamedan and Babol

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Background and objectives: Upon recent finding of a new *Gongyloema* species (*G. nepalensis*) in domestic and wild ruminants in Asia and Europe, this study aimed to investigate the rate of infection in cattle, sheep and goats slaughtered in Hamedan, and sheep and goats in Babol slaughterhouse.

Methodology: From March 2018 to June 2019 full-length esophagi were collected from 968 livestock including 384 cattle in Hamedan slaughterhouse and 584 sheep and goats (384 from Hamedan and 200 from Babol). In laboratory esophagi were inspected and the worms embedded in tissues were carefully retrieved, preserved in ethanol containing glycerin and identified morphologically.

Results: Out of 384 examined esophagi of cattle three (0.8%) harbored *Gongylonema*. Morphological characteristics nematodes were consistent with *G. pulchrum*. Parasites were found in first proximal portion (n=2) and middle portion (n=1) of the esophagi. No pathologies attributed to gongylonemiasis were found in this study. Infection was not observed in small ruminants.

Discussion and Conclusion: This study provides the first information about infection of livestock in Hamedan province with *Gongylonema*. Low infection rate of cattle and absence of infection in small ruminants of Hamedan shows that infected intermediate hosts are not abundant in the area and so risk of human infection is low. Absence of infection in sheep slaughtered in Babol might suggest that climate change and/or improvement of rearing conditions and hygiene measures led to this decrease. Further epidemiological studies with aid of molecular biology tools are necessary to find out if *G. nepalensis* does occur in Iran.

Keywords: Gullet worm, Vector-borne, Livestock, Small ruminants, Zoonoses

Invitro study of antitrichomonal activity of marine Tunicate from the Persian Gulf

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Introduction: *Trichomonas vaginalis* is causative agent of Trichomoniasis, a sexually transmitted disease with more than 143 million people worldwide that are affected by this infection. The common and first choice drug used to treatment the trichomoniasis is metronidazole that many cases of treatment failure has recently been reported. Because of the increasing drug resistance in treating trichomoniasis, finding new drug combinations is one of the priorities of this field.

Materials and Methods: Tunicates, also known as marine invertebrates belonging to the phylum Chordata, Were collected from the Persian Gulf coast, After washing, extraction was performed using percolation method by water, n-hegsan, ethanol and dichloromethan as solvents. The extracts were filtered and the filterates were dried using a rotary evaporator. Bioassay was performed using the different concentrations (20-800 µg/ml) of crude extracts and then the crude extracts were also fractionated by column chromatography and 18 fractions were separated that also their activity against *Trichomonas* trofozoites was evaluated. Identification of the chemical formula of the fractions was performed by GC-MS.

Results: The primary bioassay showed that the crude ethanolic extract at a concentration of 4 mg / ml had the most destructive effect against *Trichomonas vaginalis*. Among the 18 fractions obtained by chromatography, ethanolic fractions from 12 to 18 had the most toxic effect against *Trichomonas vaginalis* and the IC₅₀ of these 7 fractions are as follow respectively: 422 , 321, 166, 128, 111, 111, 42.90 µg/ml.

Discussion and Conclusion: The results showed that the most important chemical compounds affecting trichomonas are those metabolites found in ethanolic extract.

Among the seven fractions isolated from the ethanolic extract, the fraction number 18 with IC₅₀= 42.29 µg/ml and chemical fomula of “ phenol.2,4-bis(1,1-dimethyl ethyl) ” has the highest activity against *Trichomonas vaginalis*.

Key word: *Trichomonas vaginalis*, treatment, tunicate

Efficient and robust Nested-PCR method in detecting asymptomatic cases toward Malaria elimination program in Hormozgan province, Iran

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Introduction: Malaria is a conventional parasitic disease in the world. According to the World Health Organization (WHO) report in 2018, Malaria found in 91 countries worldwide. Due to the effects of Malaria on public health, WHO has created comprehensive planning for control, elimination and eradication of Malaria. Currently, Iran is a member of the twenty-one countries have been recognized to have potential to eliminate Malaria by the year 2020 (E2020), and the Malaria elimination program is underway in this country. In recent years, low levels of parasites and asymptomatic cases have been identified as a major challenge in the elimination program because it not be able to detect by routine Malaria diagnosis techniques, including microscopic and RDT methods. Although these methods are simple and inexpensive, they are not enough effective and sensitive to detect low levels of parasitic, mixed infections and asymptomatic malaria cases. Accordingly, the aim of this study is to use a sensitive molecular techniques (Nested-PCR) along with routine diagnostic methods to detecting asymptomatic Malaria cases.

Materials and methods: In this cross sectional study, 210 samples were randomly collected among inhabitant of high risk areas of Malaria in Hormozgan province. The asymptomatic infection rate of *plasmodium* was determined by using microscopic (by two trained personal), Rapid Diagnostic Test (at the same place of sampling) and Nested-PCR assay. Sampling was performed on individuals who had no apparent Malaria symptom.

Result: In this survey, after analysis all samples no positive malaria parasite were observed by microscopy and RDT methods. However Nested-PCR could detect three (1.4%) positive cases.

Conclusion: As it is important to discover all of the asymptomatic Malaria reservoirs to achieve malaria elimination, a sharp diagnosis technique is required. In order to successfully implement the Malaria elimination program, it is essential to diagnose all positive cases, especially asymptomatic and low parasitemia. Although microscopy and RDT are routine methods for Malaria diagnosis, however they have no enough sensitivity to detect asymptomatic cases. Therefore, diagnostic tools with high sensitivity like Nested-PCR are required for the detection of asymptomatic Malaria cases.

Keywords: Asymptomatic Malaria, *Plasmodium*, Nested-PCR

Study on *Periplaneta americana* (Blattodea: Blattellidae) fungal infections in hospitals sewer system in Esfahan City, Iran

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Objectives: American cockroaches contaminated with pathogens inside hospital manholes can be one of the major problems health care systems face. Because of their specific biological behaviors, Cockroaches can cause hospital infections in hospitalized patients and their companions.

Methods: A total of 55 American cockroaches trapped from the wall of the sewage network were transferred to the 7 major treatment centers in Esfahan city, Samples hand-captured individually in the tube and after an hour of rest at the laboratory of fungal Infections (Surface and interior) were studied.

Results: All cockroaches collected from hospitals were infected with fungi. Among the 24 (44%) female cockroaches and 31 (56%) male cockroaches, infected with 40.00% *Aspergillus niger*, 3.63% *Rhizopus*, 7.27% *Penicillium*, 5.45% *Mucor*, 38.18 % *Candida glabrata*, 14.54% *Candida viswsnstii*, 52.72% *Candida kudriavzevii* and 43.63% *Candida kluyveri* were identified respectively. The results of this study showed that *Candida krusei* had the highest prevalence among isolated fungi with 52.72% of the digestive system and *Aspergillus niger* with 40.00% of the surface of the cockroach body.

Discussion & Conclusion: Results emphasized the role played by cockroaches as potential pathogenic agents in hospital environments. Therefore, suitable management is needed in controlling this insect to prevent disease transmission in hospitals .

Keywords: American cockroach, hospital, fungal infection, Esfahan, Iran.

Is there a correlation between bruxism with parasitic infections in children?

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Background and aims: Bruxism or teeth grinding is a prevalent oral habit among children and a potential destructor of tooth and oral tissues. This behavior is reported in about 20% of children up to the age of 11 years. Bruxism is reported in about 20% of children up to the age of 11 years, although this is probably an underestimate as the condition is sometimes unnoticed by parents. This behavior causes nocturnal arousals and is classified as a sleep disorder. Episodes of bruxism last around 4 seconds and occur approximately 6 times per hour. They tend to occur in clusters throughout the night with the majority in Stage 2 and REM sleep. Multiple factors have been considered in the etiology of bruxism in pediatric patients. It is a common complaint in children with difficulties such as oral respiration, sleep apnea, tonsillar hypertrophy and psychiatric disorder in child or mother. Although in old texts of medicine had been noticed that there were a correlation between bruxism and intestinal parasitic infection, but in the new text books of psychiatry it is not suggested.

Method: In this article for accurate assessment of this correlation, we reviewed several studies which have been recently published.

Results: Two studies have been done in Iran. In one of them statistically significant relationship was observed between infection with pathogenic parasites and bruxism ($P < 0.05$). But in another study this relationship was not significant statistically. On the other hand in several studies at the different regions of world none causative relation between bruxism and intestinal parasitic infection were found.

Discussion and Conclusion: Given the fact that bruxism is not only a prevalent oral habit among children and a potential destructor of oral tissues but all of it is a major source of worries for their parents, the diagnosis of this behavior and correct treatment of it is very critical. Therefore it is worthy that pediatricians that usually visit children with noticed behavior, carefully examine the mental status of them or preferably consult with a psychiatrist. Furthermore in the cases that risk of teeth destruction is high referring of them is strictly recommended.

Molecular identification of *Blastocystis* subtypes, isolated from humans in Shiraz city, southern of Iran

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Abstract

Background: *Blastocystis* is the most common intestinal parasite in human and animal. Due to the relationship of the subtypes (STs) of *Blastocystis* with clinical manifestation in human, further studies for identification of different STs of this parasite using molecular techniques is on demand. This study was conducted for identification of different STs of *Blastocystis* isolated from human in Shiraz city, southern of Iran.

Material and Methods: Human fecal samples (n=17) that were positive for *Blastocystis* using microscopic method, were collected from hospitals of Shiraz. After DNA extraction, small subunit ribosomal DNA (SSU-rDNA) gene was amplified by the polymerase chain reaction (PCR) using Blast 505-532 and Blast 998-1017 primers. After running the PCR product using electrophoresis, the specific bands (500-bp) were isolated and sequenced. Obtained sequences were compared with published sequences in GenBank using BLAST system. Phylogenetic analysis was performed using the Maximum Likelihood method in the MEGA 5.0 software.

Results: Sequencing analysis showed 4 STs of *Blastocystis* in our samples. These STs were including ST1 (17.65%), ST2 (29.41%), ST3 (47.06%) and ST7 (5.88%).

Conclusions: ST3 was the most prevalent subtype in our study and is considered as a dominant human ST in the world. For characterization of further STs of *Blastocystis*, comprehensive molecular studies with a large number of *Blastocystis* isolates are suggested.

Keywords: *Blastocystis*, Subtype, Polymerase Chain Reaction (PCR)

Innovation in Methods for the production and maintenance of *Toxoplasma gondii* Tachyzoites of PRU strain, as a Genotype II

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Background & Aim: *Toxoplasma gondii* is apicomplexan parasite which is widely prevalent in warm-blooded animals that causes severe infections. Production and storage of tachyzoites are essential for all experimental models. In this study, Innovation in Methods for the production and maintenance of *Toxoplasma gondii* Tachyzoites of PRU strain, as a Genotype II.

Methods & Materials: Immunosuppressing method: to obtain tachyzoites, 10-15 cysts from chronically infected mice were inoculated intraperitoneally into mice that had been treated with Dexamethasone 10 mg/kg, Cyclophosphamide 36 mg/kg and Cyclosporine 18 mg/kg orally from the 5 days prior to the infection, until the tachycardia was sustained.

Pepsin digestion: the homogenate brain containing cyst was disrupted by pepsin digestion.

Mass cultivation of the tachyzoites was provided due to cell cultivation of produced tachyzoites and bradyzoites as mentioned above.

The maintenance of the tachyzoites was performed using cryopreservation (92 % FCS plus 8 % DMSO). The viability of the cryopreserved tachyzoites was evaluated by trypan blue and cell culture.

Results: In immunosuppressed mice, after 10 to 15 days from infection, tachyzoites were observed in peritoneal fluid, also bradyzoites were discovered after the cyst wall digestion by pepsin. Mass cultivation of tachyzoites and bradyzoites in Hela cell was released a lot of tachyzoites. Trypan blue staining and Hela cell culture was confirmed the correctness of cryopreservation and viability of PRU tachyzoites.

Discussion & Conclusion:

Findings of the current study suggested in vivo and in vitro culture systems to production and maintenance of *Toxoplasma gondii* Tachyzoites, PRU strain that could provide fresh viable tachyzoites as and when required.

Keyword(s): *Toxoplasma gondii*, PRU strain, Tachyzoites, Genotype II, maintenance

The Relationship between *Toxoplasma* infection in mothers and offspring gender

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Background & Aim: *Toxoplasma gondii* is one of the mostcommon parasitic protozoan in humans. It is a classical model for the manipulation hypothesis study. The behavioral changes, impairment of memory, hyperactivityand even influence human pregnancy are the manipulative activity of the *Toxoplasma*. The sex may be influenced by many factors, such as stress and immunosuppression and age of parents. In this study, we compared the genders of offspring in their *Toxoplasma*-positive and *Toxoplasma*-negative mothers.

Materials and Methods: From 2014 woman whohad been tested for toxoplasmosis, the data of 137 mothers with chronic toxoplasmosis and 137 healthy counterparts were collected from clinics and hospitalsof Shiraz city.

The sex of a offspring's, as well as the mother's age and the number of girls and boys of previous pregnancies, abortions and their gender, contact with cats, food habits, education level and place of residence,were recordedfor analysis.

Results: The number of girls was 165 (49.44%) and 136 (45.48%) in the *Toxoplasma*-positive and the *Toxoplasma*-negative mothers respectively.

The number of boys was 166 (50.15%)and 163 (54.51%) in the *Toxoplasma*-positive and the *Toxoplasma*-negative mothers respectively. In mothers with chronic toxoplasmosis, the number of girls shows thats significant correlation.

Discussion & Conclusion: In this study, a significant relationship was found between chronic *Toxoplasma* infection and secondary sex ratio. However, it is suggested that this relationship be investigated in further studies as well as in laboratory animals.

Keywords: *Toxoplasma* infection,mothers,gender, neonates, sex ratio

Application of CRISPR/Cas9 tools for *Leishmania* genome editing

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Background: The availability of *Leishmania* genome sequences and engineering tools enable reverse genetic manipulation of the parasite to study genes encoding proteins which involved in pathogenesis and/or cellular processes. Classical genome manipulation methods such as Zinc-finger nucleases and TALEN have significant limitations. Increased efficiency for the genetic manipulation of parasitic protozoa has occurred by adoption of clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 mediated gene editing.

CRISPR/Cas9 has been successfully used through different strategies to generate PFR2 gene knockout (KO) in *L. major*, miltefosine transporter KO in *L. donovani*, A2 multigene KO in *L. donovani*, BTN1 KO in *L. mexicana* and CK1.1 KO in *L. donovani*. A novel system uses parasites stably expressing hSpCas9 and T7 RNA-polymerase that are transfected with sgRNA fragment and the donor DNA cassettes. This strategy was successfully applied to *L. mexicana*, *L. major*, and *L. donovani* and in our study in *L. major*.

Materials & methods: Using this toolkit for fast and accurate genome editing, we are establishing KO *L. major* parasites lacking 2 kinetoplast genes encoding proteins that have roles in biology of *Leishmania*.

Results: The CRISPR/Cas9 gene knockout using this strategy is establishing in the lab and result of the pathogenicity study of KO parasites will be published soon.

Conclusions: Several approaches of CRISPR/Cas9 editing have been used in *Leishmania* for reverse genetic manipulation to elucidate gene functions. It is hoped that the establishment of the current approach will help in characterization of the role of kinetoplast genes in parasite survival or pathogenesis.

Keywords: *Leishmania*; Kinetoplast; CRISPR/Cas9; Knockout; Pathogenicity

Micronemes as *Toxoplasma gondii* vaccine target

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Abstract

Toxoplasmosis is a cosmopolitan zoonotic infection, caused by a unicellular protozoan parasite known as *Toxoplasma gondii* (*T. gondii*) that belongs to the phylum Apicomplexa. More than one-third of the human population are seropositive worldwide. Due to the high seroprevalence of *Toxoplasma gondii* infection worldwide, the resulting clinical, mental, and economical complications, as well as incapability of current drugs in the elimination of parasites within tissue cysts, the development of a vaccine against *T. gondii* would be critical. In the past decades, valuable advances have been achieved in order to identification of vaccine candidates against *T. gondii* infection. Microneme proteins (MICs) secreted by the micronemes play a critical role in the initial stages of host cell invasion by parasites. In this review, we have summarized the recent progress for MIC-based vaccines development, such as DNA vaccines, recombinant protein vaccines, vaccines based on live-attenuated vectors, and prime-boost strategy in different mouse models. In conclusion, the use of live-attenuated vectors as vehicles to deliver and express the target gene and prime-boost regimens showed excellent outcomes in the development of vaccines against toxoplasmosis, which need more attention in the future studies.

Keywords: *Toxoplasma gondii*, Vaccines, Microneme, Immune responses, Adjuvant

Evaluation of drug sensitivities of *Leishmania major* and *Crithidia* spp. coinfection isolated from the human lesion.

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Objective: *Leishmania major* and *Leishmania tropica* have been considered the main agents of cutaneous leishmaniasis in Iran. Furthermore, there are some reports regarding the co-infection of *Crithidia* spp. with *Leishmania* parasites in cutaneous lesion especially in the resistance form to glucantime. The need for treatment of these patients harboring both microorganisms made us design this study.

Methods: In this study, the anti-*leishmania* effects of glucantime, amphotericin B and Paromomycin on promastigotes of *Leishmania major* and *Crithidia iranica* after 24 and 48 h in in vitro were investigated. The toxicity of these drugs was also assessed on mouse J774 cells then the effect of these treatments tested on intracellular amastigote forms and compare with promastigote forms.

Results: This study showed that different concentrations of glucantime, amphotericin B and Paromomycin have a dose-dependent inhibitory effect and significantly ($P < 0.05$) inhibited the growth of promastigote of both parasites.

In macrophage amastigotes model, drugs significantly reduced the growth rate of the parasites and were more susceptible to the antileishmanial drugs compare with promastigote forms. Due to the low growth of *Crithidia* amastigotes in macrophages, it was not possible to test the sensitivity of the drug using this method.

Conclusion: The present study demonstrated that amphotericin B and paromomycin in in vitro inhibited the growth of *Crithidia* and *Leishmania major* and had no cytotoxicity effect on macrophge cells.

Keywords: *Leishmania major*, *Crithidia iranica*, MTT assay, Drug sensitivity

Therapeutic efficacy of nanocompounds in the treatment of cystic and alveolar Echinococcosis: challenges and future prospects

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Abstract

Echinococcus granulosus sensu lato and *E. multilocularis* are the causative agents of life-threatening cystic and alveolar echinococcosis (CE and AE), respectively, which lead to serious public health concerns across the globe. Benzimidazoles (BMZs) are the drugs of choice for the treatment of human CE and AE. Presently, the chemotherapeutic failures of BMZs against CE and AE are due to their low aqueous solubility, poor absorption, and consequently their erratic bioavailability. Among BMZ compounds used for CE/AE treatment, Albendazole (ABZ) and Mebendazole (MBZ) are the only drugs licensed for the human use. Nevertheless, the administration of these BMZs for a long period of time leads to undesirable adverse effects. Therefore, there is an urgent need for designing new formulations of BMZs with increased bioavailability. To bridge these therapeutic gaps, nanoparticle enantiomers of ABZ and drug delivery systems based on nanostructured entities currently provide an interesting new formulation of already existing drugs to improve the pharmacokinetic effects of BMZs. This study provides an overview of the tested nanocompounds against *E. granulosus* and *E. multilocularis*, including their effective dose, type of nanoparticles (NPs), assay setting, and therapeutic outcomes. This review suggests that BMZ derivatives loaded in NPs can significantly improve the scolicidal and cysticidal activities compared to single BMZ. Moreover, BMZs-loaded polymeric NPs show a tendency to increase mortality rate against protoscoleces and microcysts compared to metallic formulations, nanoemulsions, lipid nanocapsules, solid lipid NPs, liposomes, and nanocrystals. In the future, the use of the newly structured entities, attained by bridging ligands to the modified surface of NPs, as well as the electromagnetically produced nanodrugs would be helpful for developing fine-tuned formulations as an alternative to the already existing drugs against these neglected parasitic infections.

Keywords: Nanocompounds, Scolicidal agents, Cystic Echinococcosis, Alveolar echinococcosis, Benzimidazoles.

Assessment of the global pattern of genetic diversity in *Echinococcus multilocularis* inferred by mitochondrial DNA sequences

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Abstract

The aim of this review was to assess our current knowledge on phylogeography and global genetic structure of *Echinococcus multilocularis* populations originating from rodents, wild canid hosts, and human. Six bibliographic databases were searched from 1990 to 2017, identifying a total of 110 publications. The cytochrome c oxidase subunit 1 (cox1) and cytochrome b (cytb) sequences of *E. multilocularis* from Asia, Europe, and North Americas were analyzed to estimate the diversity and neutrality indices, and genetic differentiation. A total of 69 (cox1, 36.7%) and 16 haplotypes (cytb, 19.2%) were grouped into various geographical clades. A parsimonious haplotype network demonstrated a star-like feature with haplo-groups Em2 (Asia: 36%), Em105 (Eastern Tibetan plateau: 4.8%), Em46 (Europe: 9.1%), Em73, (Europe: 2.7%) and Em92 (North Americas: 4.3%) as the most common haplotypes. A relatively high level of genetic diversity was detected in rodent-derived *E. multilocularis* isolates (Haplotype diversity: 0.944), wild canids (Hd: 0.912), and human origin (Hd: 0.704). The highest number of haplotypes (n=59) and the highest haplotype diversity (0.969) were identified in the Asian and European populations, respectively. Cladistic phylogenetic tree indicated the European clade has a sister relationship with the Asian clade. However, some North American haplotypes were assigned to the European clade together with haplotypes from Poland. The statistically significant F_{st} values indicated that *E. multilocularis* populations of Asian-European, Asian-North American, and European-North American origins were genetically differentiated (F_{st} : 0.22624 to 0.43059). An occurrence of distinct parasite populations suggests that *E. multilocularis* derived from glacial refugia have been plausibly sustained by indigenous hosts during the Pleistocene Epoch.

Keywords: *Echinococcus multilocularis*, Intraspecific genetic diversity, Phylogeography, Cytochrome c oxidase subunit 1, Cytochrome

Comparison of Prevalence of *Toxoplasma* Infection between Parkinson Patients and Equal Healthy Individuals

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Background & Aim: The relationship among *Toxoplasma gondii* infection and a lot of diseases has been proven. However, researchers have not reached a certain connection between Parkinson's disease and *Toxoplasma gondii* infection. The aim of this study is to evaluate relationship between the prevalence of *Toxoplasma gondii* infection and Parkinson patients.

Material and Methods: A number of 240 cases including 120 Parkinson patients with the average age of 63.21 years and healthy people with an average age of 63.99 years participated voluntarily. Also, all individuals signed the Helsinki form for ethical considerations. After separation of blood serum samples, the samples were investigated for the presence of *Toxoplasma gondii* IgG using ELISA test.

Results: The results showed that the prevalence of *Toxoplasma* was 25.8% and 15% in patients with Parkinson's disease and the control group respectively, which had a significant relationship ($p=0.037$). Also, IgG positive individuals had less severity of Parkinson clinical signs.

Discussion & Conclusion: In this study, the prevalence of *Toxoplasma* was significantly in Parkinson's patients, so that infection with *Toxoplasma* can be considered as a risk factor for Parkinson's disease. Although, *Toxoplasma gondii* may be a potential cause of Parkinson's disease, it decreases clinical signs of Parkinson in chronic form due to parasite dopamine production.

Keywords: Parkinson disease, *Toxoplasma gondii*, ELISA test

Synthesis and in Vitro Study of Antitrichomonal Activity of Chalcone Derivatives on *Trichomonas gallinae* trophozoites

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Background & Aims: Chalcones are open-chain precursors for biosynthesis of flavonoids and isoflavonoids and occur mainly as polyphenolic compounds. Chalcones display enormous number of biological activities, e.g., anti-inflammatory, anti-oxidant, anti-protozoal, and etc. *Trichomonas gallinae* is the relevant agent of avian Trichomonosis which existence of resistant strains of *T. gallinae* to the medications has been observed. In that respect, we have attempted to evaluate the anti-Trichomonal activity of (1E,4E)-1,5-bis(3-nitrophenyl) penta-1,4-dien-3-one as an effective derivative of chalcones.

Methods: A solution of NaOH was added dropwise to a flask containing acetone (0.02 mol) and benzaldehyde (0.04 mol) in ethanol. (1E,4E)-1,5-bis(3-nitrophenyl) penta-1,4-dien-3-one precipitate. Different concentration effects of this compound were determined after 24, 48, and 72 hours on *T. gallinae* (1×10^7 trophozoites/ml) which were cultured in a Hollander's modification of TYM medium.

Results: The results show that the different concentrations of chalcone were able to reduce the viability of *T. gallinae* trophozoites. The alive trophozoites were decreased remarkably with enhancing the concentration of chalcone and exposure time. The different concentrations of chalcone exhibited different degrees of growth inhibition activity on *T. gallinae*. In the higher concentrations of chalcones (10 and 5 mg/ml) 48h after adding trophozoites to the dilutions, the motile of trophozoites were decreased and growth inhibitory effect was 100%.

Conclusions: In this study (1E,4E)-1,5-bis(3-nitrophenyl) penta-1,4-dien-3-one was synthesized and evaluated its inhibitory activity against *T. gallinae* trophozoites. According to the results obtained, it can be concluded that the synthesized compound has an anti-trichomonal activity for treating metronidazole-resistant isolates of *T. gallinae*.

Keywords: Chalcone derivatives, *Trichomonas gallinae*, Drug resistance, SEM.

Relationship between Toxoplasmosis and Autism: A Systematic Review and Meta-Analysis

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Background: *Toxoplasma gondii* (*T. gondii*) is a foodborne parasite that is investigated in many psychiatric diseases, such as autism spectrum disorders (ASD). Therefore, a systematic literature review was conducted searching 7 electronic databases on the prevalence of *T. gondii* antibodies among autism patients.

Methods: The current study involved sensitivity analysis, meta-regression, subgroup analysis, publication bias test, and quality assessment of studies.

Results: On the basis of the findings, the OR of latent *Toxoplasma* infection 1.93 (95% CI: 1.01-3.66) was associated with ASD risk. However, there was no relationship between acute infection and ASD 0.39 (95% CI: 0.18-0.87). The obtained results of Begg's and Egger's tests showed no publication bias ($p=0.851$ and $p=0.297$, respectively). The sensitivity analysis confirmed robust and stable estimates with a significant level of heterogeneity ($I^2=78.1\%$, $p<0.000$). Of the investigated patients' characteristics, only the gender variable was analyzed, indicating the combined ORs of 2.63 (95% CI: 0.29-23.63) in female and 2.62 (95% CI: 0.94-7.30) in male participants.

Conclusions: This study showed that toxoplasmosis plays an important role as a risk factor for autism. However, further prospective investigations are highly recommended to illuminate the developmental pathways to this disorder and provide new strategies for the prevention and treatment of this disease.

Keywords: *Toxoplasma gondii*, toxoplasmosis, autism, autism spectrum disorder, ASD

Developing a simple cultivation of *Plasmodium falciparum* erythrocytic stages at high parasitemia

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Background: Malaria caused by *Plasmodium falciparum* is a major health problem in about 100 tropical countries infecting 219 million people leading to approximately 435000 related death in 2017, mostly among children, pregnant women and non immune visitors to malarious areas. For the control and eradication of Malaria, deep understanding of its biology, immunology, biochemistry and pharmacology is essential and for aiming this purpose continuous culturing of *P.falciparum* is absolutely critical.

In recent years research on the most pathogenic Malaria parasites has expanded because of Trager and Jensen demonstrated in 1976 that the erythrocytic stages of *Plasmodium falciparum* could be cultured in vitro. Since that time efforts have been expended in trying to develop and simplification of the original methods. In this study, we present modified simple method of in vitro continuous culture of *P. falciparum* at high parasitemia.

Result: We improved standard method in order to achieve a practicable and reproducible in vitro Malaria continuous culture with an amazing resulting parasitemias about 60% just within 7-10 days.

Conclusion: For the first time in Iran, we established a simple continuous modified protocol in Malaria culturing to produce high parasitemias in duration of 7-10 days which was nearest to in vivo system. This method can be a starting point that facilitates investigations which led to increase of our knowledge in Malariology. Drug screening and resistance mechanism, vaccine development, pathogenesis surveys, molecular and cellular biology and genetics are areas have been impressed by use of in vitro continuous cultivation of *P. falciparum* parasites.

Anti Leishmanial Effect of *Plantago psyllium* (ovata) and White Vinegar on *Leishmania major* Lesion in BALB/C Mice

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Abstract

Background: *Leishmania major* causes rural leishmaniasis in some parts of Iran and for treatment the currently used drug is Glucantime by injection on the site of the ulcers. The aim of this study was to evaluate the anti leishmanial effect of topical *Plantago psyllium* (ovata) and white vinegar in *Leishmania major* infected BALB/C mice.

Material and Methods: In this experimental study, 30 infected BALB/C mice divided into 5 groups. All mice had leishmanial ulcer that confirmed by microscopic examination. Group 1: treated with the combination of ovata powder and white vinegar. Group 2: treated with the Glucantime. Group 3: treated with the white vinegar. Group 4: treated with the combination of ovata powder and water. Group 5: without any treatment. Lesion sizes measured weekly and final smears prepared in the last day for microscopic examination.

Results: The results showed a significantly ($P < 0.05$) smaller lesion size in the mice in the treated groups especially in Glucantime and vinegar groups than in the mice in the control group. Anti leishmanial effect of vinegar groups is the same as Glucantime and ovata effect is less than theirs.

Conclusion: It seems the most anti leishmanial effect is related to vinegar and supported by *Plantago*. However, the route of treatment with this combination is very simple and painless in comparison with injection. So by addition study scientists could design the effective and more easily using drugs.

Keywords: *Leishmania major*, *Plantago psyllium*, Iran

Genotyping of *Echinococcus granulosus* Isolates From human and livestock based on Sequencing of COX-I Gene in Bushehr Province

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Background: Echinococcosis is a helminthic disease caused by the tapeworms *Echinococcus* spp that occurs in a number of herbivorous, omnivorous and carnivorous animals. This disease is an important public health problem in some parts of Iran. Depending on the infected host, *Echinococcus* can cause serious morbidity, livestock production losses, reduction in carcass weight, fertility and milk production, and possibly death. The aim of this study was to genotyping of *E. granulosus* isolates from human and livestock in Bushehr province.

Materials and methods: In this study, isolates of 25 hydatid cysts were collected from the liver, lungs, heart and spleen of intermediate hosts (human, sheep, cattle, goat, camel and buffalo). DNA was extracted and examined by polymerase chain reaction (PCR) and sequencing of Cytochrome C Oxidase I (COX-I) gene.

Results: The results of 25 isolates sequencing analysis, sign of existence and identification of 3 genotypes: G1 (n=15), G3 (n=7) and G6 (n=3) among all samples in this region.

Conclusion: The molecular results showed that the common genotype in Bushehr is sheep strain (G1 genotype), which occurs in human, cattle, sheep and goat populations. In conclusion, these results may have important implications for Echinococcosis control in Bushehr province.

Keywords: Echinococcosis, Genotyping, Iran

Epidemiologic Survey ON Blood Parasite OF Sheep from Golestan Province Northe OF IRAN 2017

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ABSTRACT

Babesia, *Theileria* and *Anaplasma* are the most hemoparasites that transmitted with thick and find in many animals as ruminants that cause many economic damages and fatal diseases. Clinical signs are fever, loss weight, icterus and hemogluninuria. Our study was investigating in 500 sheep as hemoparasite from blood smear that extract from suspected sheep from cities in Golestan province during 2017. According our knowledge prevalence of hemoparasites demonstrate the 17.4% Babesiosis, 9.2% Theileriosis and 7.4% Anaplasmosis, repectively.

Keywords: *Babesia*, *Theileria*, *Anaplasma*, sheep, Iran

Fe₃O₄@bio-MOF Nanoparticles Combined with Artemisinin, Glucantime®, or Shark Cartilage Extract as Novel Treatments for Leishmaniasis

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Introduction: Cutaneous leishmaniasis is the most important health problem in various countries especially in Iran. Efforts have been made to eliminate treatment failures as an impediment to effective anti-*leishmania* chemotherapy.

Methods: In the present study, we examined effects of Fe₃O₄@bio-MOF nanoparticle plus artemisinin, glucantime, and shark cartilage extract on Iranian strain of *L. major* (MRHO/IR/75/ER) in vitro and in vivo. Promastigote and amastigote assays as well as flow cytometry were conducted at the presence of 3.12- 400 µg/mL of the drugs. According to invitro IC₅₀ results, the combinations of 12.5 µg/ml Fe₃O₄@bio-MOF+ 25µg/ml artemisinin, 12.5 µg/ml Fe₃O₄@bio-MOF+ 200 µg/ml Glucantime, and 12.5 µg/ml Fe₃O₄@bio-MOF+ 0.5 ml of 20 mg/kg of shark cartilage extract were used to treat Balb/c mice. During and at the end of the treatment of Balb/c mice, the lesion sizes were measured and parasite loads, IFN-γ, and IL-4 levels were evaluated at the end of the treatment. The

Results: IC₅₀ of Fe₃O₄@bio-MOF-Artemisinin, Fe₃O₄@bio-MOF-Glucantime, and Fe₃O₄@bio-MOF—Shark cartilage extract on promastigotes were 12.58± 0.1, 235± 0.1, and 18.54± 0.2 µg/ml, respectively. These results on uninfected macrophages were 243.84± 0.1, 317.28± 0.2, and 256.54± 0.2 µg/ml, respectively, and on amastigotes were 10.32± 0.1, 187± 0.1, and 338± 0.2 µg/mL, respectively. The apoptosis percentage of these combinations were 32.54%, 20.59%, and 15.68% in promastigotes and 15.68%, 12.84%, and 3.51% in infected macrophages, respectively with no toxicity on uninfected macrophages. In vivo results showed that all drug combinations were effective on the healing of cutaneous leishmaniasis (p<0.05), but Fe₃O₄@bio-MOF/Artemisinin combination was more effective on healing the lesions than other combinations (p=0.001). For Fe₃O₄@bio-MOF/Artemisinin combination, the reduction in parasite load in spleen, calculated to be 81%, was higher than other groups (p<0.05). IFN-γ level in Fe₃O₄@bio-MOF+Artemisin group was significant and higher than other groups (p<0.05).

Conclusion: This study suggests that Fe₃O₄@bio-MOF combined with artemisinin, glucantime, or shark cartilage extract especially Fe₃O₄@bio-MOF+Artemisin can be considered as an anti-*leishmania* combination therapy in cutaneous leishmaniasis induced by *L. Major*.

Keywords: Fe₃O₄@bio-MOF nanoparticle, Artemisinin; Glucantime; Shark cartilage extract, *Leishmania major*

The Investigation of Parasitological Contaminations to Evaluate Zoonotic Diseases in Goldfishes (*Carassius Auratus*) Presented in Urmia

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Introduction: Goldfish is one of the most popular ornamental fish in the world and has made up a small part of the Norouz. The most concerned issue for fish breeders and purchasers is the parasitic diseases and possibility of their transfer to human. The purpose of this paper is to investigate the parasitic contaminations in goldfishes to assess zoonotic diseases presented in the Urmia.

Methods: Obtained number of 35 fishes out of major centers of fish distribution in Urmia, in March 2019. Original breeding sites of these fishes were located at Saqalaksar and Sangar Dam, in Gilan State. Sampling performed on the external organs and tissues (from skin, fins, gills and eyes), and internal organs (from ventricular cavity and digestive tract). Sampling, staining and diagnosis of parasitic specimens were carried out using standard parasitological methods and parasites detection keys.

Result: In this study, there was no any zoonotic parasite in fish. Skin with 28.57% contamination, highest and gill with 2.85% showed the least tissue contamination. Protozoan parasites with 58.33% were the most important cause of parasitic infestations and the genus *Trichodina* sp. with 33.33% was more than other parasite's species.

Discussion and Conclusion: Primary reproduction sites of the samples were placed in pond condition and it's the most important factor in parasite contaminations of these distribution centers. The lack of hygiene supervision, reproduce fishes in different places, collect and keep them in one place, will increase the possibility of parasitic infestations.

Keywords: Goldfish, Parasitic Contamination, Zoonosis, Urmia

The First Report of *Ascocotyle (Phagicola) longa*, Zoonotic Trematode (Digenea:Heterophyidae) Infected *Chelon saliens* (Mugilidae) in the Caspian Sea, Iran

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Ascocotyle (Phagicola) longa Ransom, 1920 metacercariae, as the fish-borne zoonotic trematodes were reported from Iranian waters of the Caspian sea for the first time. Sharpnose mullet fish, *Chelon saliens* (Risso, 1810) were caught by means of beach seine fishing in April 2017 and 2018, immediately deep frozen. All specimens were transferred to the laboratory of ShahidBeheshti University. After biometry evaluation, a parasitological survey implemented by stereo and light microscopes on internal organs. The highest values of prevalence (16%), mean intensity (6 ± 2.82) and mean abundance (1 ± 2.4) of the infection were found in April 2017 from Anzali location. Actually, *A. (Phagicola) longa* is a widespread fish-borne zoonotic parasite observed in the Caspian Sea that is one of the causative agents of human heterophyiasis with the potentially great public health impact.

Keywords: *Ascocotyle (Phagicola) longa*, Metacercariae, Parasite, Mugilidae, Caspian Sea

The Correlation between *Toxoplasma gondii* in Fection and Autoimmune Diseases Such as Type I Diabetes and Multiple Sclerosis

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Background: Autoimmune disorders such as Type I diabetes and multiple sclerosis (MS) may be stimulated by induced immune system components due to infectious disease. This study was undertaken to aim whether *Toxoplasma* infection is correlated to the diseases.

Material and Methods: 182 sera samples were collected from type I diabetic subjects (n=91) and control group (n=91). The individuals had less than 30 years and the number of age range in the groups was identical. Moreover, 130 patients suffering from MS and 130 healthy controls voluntarily participated in this study. The sera were examined for IgG and IgM antibodies by ELISA and compared with epidemiological factors such as age, sex, family history.

Results: The highest frequency of diabetes was belonged to 6-10 and 11-15 years groups ($P=0.17$). Toxoplasmosis rate in diabetic and control groups was 28.6%, 7.7% respectively ($P_{\text{value}}=0.001$). A significantly positive family history of diabetes was observed between diabetic patients (31 cases, 34.1%) and control group (3 cases, 3.3%) ($P_{\text{value}}=0.01$). Interestingly, IgG positivity was seen in 13 case (41.9%) of patients with positive family history of type I diabetes and 13 case (21.7%) of subjects with no positive family history of type I diabetes ($P_{\text{value}}=0.04$). Moreover, 35.4% of MS patients were seropositive to *Toxoplasma* whereas 13.8% of control group had antibodies against *Toxoplasma* ($P_{\text{value}}=0.001$). A significant difference was observed between genders of MS patients (females=58.8% and $P_{\text{value}}=0.03$).

Conclusions: Our results suggest a significant link between *T.gondii* infection and MS and type I diabetes. However, more investigations seem necessary.

Keywords: *Toxoplasma gondii*, Autoimmune diseases, Type I diabetes, Multiple sclerosis, ELISA

5-oxo-Hexahydroquinoline Derivatives: Synthesis, Molecular Docking and Their in vitro and in vivo Evaluation on *Toxoplasma gondii*

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Background: Apicoplast, an intracellular organelle which is essential for *Toxoplasma* survival and its pathways of biogenesis are sought after as promising drug target. The aim of this study was to evaluate the effects of 5-oxo-hexahydroquinoline compounds as inhibitors of apicoplast Enoyl-Acyl Carrier Protein Reductase (ENR) on *Toxoplasma gondii*.

Material and Methods: At first, compound pyridin-3-ylmethyl 4-(4-methoxyphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (C1), pyridin-3-ylmethyl 4-(4-chlorophenyl)-2-methyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (C2), pyridine-3-ylmethyl 2-methyl-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (C3) are synthesized and then tachyzoites of RH strain were exposed to various concentrations (1-64 µg/ml) of the compounds for 2 hours. The vitality of the parasite was assessed using flowcytometry and propidium iodide staining. To evaluate chemotherapy effect of C1, the tachyzoites were inoculated to four BALB/c mice groups (N=5) and then the different concentrations of the compounds were gavaged to them. Binding affinities of the synthesized 5-oxo-hexahydroquinolines into ENR enzyme active site were investigated using AutoDockVina software. Docking was performed using Lamarckian Genetic Algorithm with 100 runs.

Results: Flowcytometry results showed compound C1 had the highest mortality effect (IC₅₀ = 6.67 µg/ml). No significant difference was seen in the longevity of treated mice comparing the control group. The docking study revealed that C1 and C2 were able to inhibit the ENR enzyme.

Conclusion: Our findings demonstrate the mortality effects may not be due to ENR enzyme inhibition. Based on little effect of this compound in chemotherapy, it seems that the oral administration is not satisfactory or the compounds convert to other metabolites. Further studies are needed other administration of the compounds to enhance the optimal effects.

Keywords: Apicoplast, Enoyl-Acyl Carrier Protein Reductase (ENR), *Toxoplasma gondii*, 5-oxo-hexahydroquinoline

Prevalence of *Giardia lamblia* Infection in Patients Referred to Besat Clinic in Kerman City in 2018

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Introduction & Objective: Nowadays, parasitic infections are one of the most common and a major health problem throughout the world, particularly in developing countries. The current study aimed to determine the prevalence *G. lamblia* infection in patients referred to Besat clinic in kerman city in 2018

Materials & Methods: This cross sectional study was conducted on 383 patients referred to Besat clinic in kerman city. The samples were transferred to the Parasitology Laboratory of kerman Medical School. They were examined by formalin-ether concentration technique. The resulting was studied by light microscope. The samples were then examined by ELISA technique. The results were analyzed with SPSS, and chi-square (X²) test.

Results: Out of the 3⁸³ patients referred 32.1% were male and 67.9% female.

Totally, ⁹ (2.34%) samples were infected with *G. lamblia*. 12 (3.13%) positive cases were identified by ELISA technique.

Conclusions: The results of the study showed a prevalence of *Giardia lamblia* infection. Therefore it is necessary to promote the public health awareness, in order to reduce the frequency of parasitic infections. Based on the results of this study, ELISA method is more accurate and faster than other methods for diagnosis of *G. lamblia* infection.

Keywords: Prevalence; ELISA; *Giardia lamblia*; Kerman.

Apolipoprotein A-I: A Possible Facility for *Leishmania* Parasites to Escape Immune System

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Both innate and adaptive immune responses are needed to eliminate *Leishmania* parasites in human. Trypanolytic factor (TLF) contributes to the innate immunity against *Leishmania* parasites and is more composed of apolipoprotein A-I (APO A-I). According to our recent proteomic research, APO A-I is an immunoreactive protein in amastigotes of *Leishmania infantum*. Accordingly, we suggest that *Leishmania* parasites should use APO A-I to escape from the immune responses by means of two mechanisms; molecular mimicry and anti-inflammatory effect of APO A-I.

Leishmania parasite is able to express the parasitic antigens that are similar to the components of the host which is referred to as molecular mimicry. *Leishmania* parasites use this mechanism to induce tolerance in the host immune system. Thus, it can be suggested that *Leishmania* parasites produce and express the APO A-I to bypass and escape from TLF as a component of the innate immune responses. As mentioned, TLF is predominantly composed of APO A-I, so expression and up-regulation of APO A-I in *Leishmania* parasites probably refer to the chromosomal adaptation property of this parasite against TLF.

Also, APO A-I is an anti-inflammatory agent to suppress the activation of the neutrophils and inhibit the endothelial expression of adhesion molecules. Furthermore, this protein inhibits the production of interleukin-1 β and tumor necrosis factor- α . Again, this mechanism also supports our idea as to the role of APO A-I in *Leishmania* parasites to bypass the innate immune system. However, further in vitro experiments are needed to reveal the exact functions and mechanisms of APO A-I in the immunity and pathogenicity of *Leishmania* parasites.

Keywords: Apolipoprotein A-I, *Leishmania*, Innate immunity system

High Potency of Ozone Gas to Inactivate the *Echinococcus granulosus* protoscoleces

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Background: In medicine, ozone therapy is effectively used in a broad spectrum of diseases. Reviews have shown that ozone gas demonstrates potent antimicrobial effects against a wide range of pathogenic microorganisms such as oral bacteria, fungi, viruses and parasite even in resistance strains. The present investigation was model designed to assess the protoscolicidal effects of ozone gas on hydatid cysts protoscoleceson in vitro and ex vivo.

Methods: Hydatid cyst protoscoleces were acquired from sheep livers that were slaughtered at Kerman slaughterhouse, Iran. The viability of protoscoleces was assessed by the eosin exclusion examination after exposure with ozone gas after 1 to 14 minutes in vitro and ex vivo.

Results: In this study, in vitro assay showed that ozone gas at the concentration of 20 mg/L killed 85 and 100% of hydatid cyst protoscoleces after 4 and 6 min of treatment, respectively. However, in the ex vivo analysis, needing a more time to confirm a potent protoscolicidal activity; so that ozone gasafter exposure time of 12 min, approximately killed 100% of protoscoleces within the hydatid cyst.

Conclusion: In conclusion, the findings of the present study showed that ozone gas at low concentrations (20 mg/L) and short times (4-6 min) might be used as a novel protoscolicidal drug for use in hydatid cyst surgery. However, more clinical surveys are mandatory to discover precise biological activity of ozone gas in animal and human subjects.

Keywords: Protoscoleces, in vitro, ex vivo, *Echinococcus granulosus*, scolicidal

mmu-miR-27a as a candidate miRNA which targets Inflammatory cytokines involved in *Leishmania major*-infected macrophage

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Introduction and Objectives: miRNAs as small non-coding RNAs play an important role in regulating immune responses. Computational approaches for miRNA target prediction can help to select the best candidate miRNAs which have a role in inflammatory cytokine modulation in infectious diseases. The aim of this study was to select appropriate miRNA that targets inflammatory and anti-inflammatory cytokines involved in *Leishmania major*-infected macrophage.

Material and methods: miRNAs targeting key inflammatory and anti-inflammatory cytokines (IL-2, TNF, IFN γ and IL-10, TGF β 1 respectively) involved in leishmaniasis have been predicted using Targetscan, mirvestigator, mirwalk databases, and GEO databank.

Results: From all predicated miRNAs, mmu-miR-27a received the highest score. So that, it targets IL-10, IL-2, IFN γ , TNF and TGF β 1 with 95, 94, 93, 87 and 85 context++ score using Targetscan database respectively. It also targets IFN γ , TNF, IL-2 and IL-10 in 9, 7, 6 and 6 algorithms by mirwalk database respectively. Analysis of GEO databank showed the downregulation of miR-27a in *Leishmania major*-infected macrophage.

Discussion and conclusion: miR-27a is a candidate for further analyses in Leishmaniasis. Due to different roles of inflammatory and anti-inflammatory cytokines in the immunopathogenesis of *Leishmania* infection, manipulating miRNAs using miRNA mimic or inhibitors as cytokine regulator may be useful for control of infection.

Keywords: miRNA, Bioinformatics database, inflammatory cytokine, *Leishmania*

Phylogenetic analysis of *Hymenolepis nana* isolates from human based on ITS2-rDNA gene marker

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Background: *Hymenolepis nana* is a common worldwide-distributed cyclophyllidean enteric parasite of human, leading to severe infection in immunodeficient patients. Due to the presence of a morphologically similar cestode, namely *Rodentolepis nana* in rodents, the molecular study of this parasite is a subject of importance. In the present study, isolates of *H. nana* with human origin were phylogenetically analysed.

Methods: Genomic DNA of *H. nana* eggs were extracted from stool samples of two infected patients. Both patients were residents of Iran, but one of them was originally from Afghanistan. PCR amplification of ITS2-rDNA region was performed, and products were sequenced. Phylogenetic analysis was carried out using MEGA 7.0 software.

Results: Blast analysis showed that the present two isolates of *H. nana* had 100% homology with each other and with the isolates of *H. nana* of human and *Rhombomys opimus* from Iran, *Rattus norvegicus* and *Mus musculus* from Japan and *Rattus rattus* from India. Phylogenetic analysis indicated that the current isolates were placed in the same branch of *H. nana* isolates from other areas of the world, clearly separating from *Hymenolepis diminuta*.

Conclusion: Based on ITS2-rDNA phylogenetic analysis, *H. nana* isolates are distinctive from *H. diminuta*, but identical to each other irrespective of their human or rodents host of origin.

Keywords: *Hymenolepis nana*, human, ITS²-rDNA, Iran

Awareness, Attitude and Performance of Educational and Administrative Personnel in the Schools of Golestan University of Medical Sciences about the Transmitting of Parasites via Food and Animals

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Introduction and objectives: Identifying influential factors in the transmitting of parasites by food and animals plays a significant role in the training and raising the level of community health. This study aimed to determine the awareness, attitude and performance of educational and administrative personnel in Schools of Golestan University of Medical Sciences about the transmitting of parasites via food and animals in May 2019.

Materials and Methods: In this cross-sectional study, the data were obtained through a relevant questionnaire. The studied subjects were 170 of 206 educational and administrative personnel in the Golestan University of Medical Sciences that they had filled the questionnaires. Data were analyzed through descriptive statistics, Student's t, Chi-square and Fisher's exact tests employing SPSS software version 22.

Results: The average scores of knowledge and attitude of individuals were 36.46 ± 4.65 , 7.35 ± 1.70 , respectively. Participants received 66.8% of the maximum knowledge score and 81% of the maximum attitude score. Of the 170 people, 21 (12.4%) had pet dogs, 4 (2.4%) kept cows and sheep at home. 96.5% of cases used raw vegetables. The average performance score for washing was 2.92 ± 0.88 . Overall, the studied staff received 73% of the maximum performance score. There was a significant relationship between knowledge and attitude with age ($p = 0.001$), educational degree ($p = 0.001$) and work experience ($p = 0.001$).

Conclusion: Awareness and attitude of educational and administrative personnel in Schools of Golestan University of Medical Sciences were good.

Keywords: Awareness, Knowledge, Attitude, Performance, Personnel, Parasites, Schools of Golestan University.

The Frequency of Canine Visceral Leishmaniasis In Mashhad, Iran

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Background: Zoonotic visceral leishmaniasis is caused by *Leishmania infantum* in the Mediterranean region and the Middle East; dogs are the main reservoirs of the disease. The aim of present study was to determine the frequency of canine visceral leishmaniasis in symptomatic dogs in Mashhad area.

Methods: The blood samples were collected from fifty dogs with one or several clinical signs taken from the cephalic vein by syringe. The blood samples were transferred into plain tubes and centrifuged at 800×g for 8 min. The obtained serum was stored at -20 °C and examined using rapid immunochromatography test.

Results: Among clinically suspected dogs, 26% (13/50) of sera samples were positive for *L. infantum* by rapid immunochromatography test. A total of 50 sera, 29 male and 21 female symptomatic dogs were examined by rapid immunochromatography test. The frequency rate in male and female dogs was not significantly different. The seroprevalence rates in guard, owned and stray dogs were 25% (4/16), 31% (6/19) and 20% (3/15), respectively. The frequency rates for visceral leishmaniasis in small and large breeds of studied dogs were 31% (6/19) and 22% (7/31), respectively. Significant difference was not observed between age and breed of the studied dogs. The rapid immunochromatography test had low sensitivity (26%).

Inclusion: The rapid immunochromatography test could be used to detect *L. infantum* in infected clinically dogs. It needs more investigation about the sensitivity and specificity of rapid immunochromatography test.

Key word: *Leishmania*, dog, immunochromatography test

Treatment Failure Cases in Cutaneous Leishmaniasis: A Retrospective Study in Iran from 2008 -2017

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Background: Cutaneous leishmaniasis (CL) is one of the most important infectious diseases in eastern Mediterranean countries including Iran. Pentavalent antimonials have been used successfully worldwide for the treatment of leishmaniasis since the first half of the 20th century, but the last 10 to 20 years have witnessed an increase in clinical resistance.

Methods: This is a retrospective study. All patients with lesion from different parts of Iran who referred to the laboratory of leishmaniasis, School of Public Health, Tehran University of Medical Sciences (TUMS), during 10 years (2008–2017), were assessed in this study. Patient characteristics (gender, age and place of residence), number, microscopic examination results, type and location of the lesions, comorbidities and type of treatment were recorded and analyzed.

Results: During this period, totally 1480 suspected CL patients referred to laboratory of leishmaniasis. 655 cases of all suspected CL samples (70.8%) were positive microscopically. The failure rate for patients treated with a minimum of three complete treatment periods with anti-leishmaniasis compounds were 12 cases (1.83%). There were no associations between age, sex, weight, the route of administration, the number and size of lesions.

Conclusion: Pentavalent antimonial (SbV) components are the first line of chemotherapy against leishmaniasis. However, the medicine has been found ineffective. In the present study, Treatment failure were seen in 1.83 % of CL patients who treated with Meglumine antimonite (Glucantime®).

Keywords: Cutaneous leishmaniasis, Glucantime, Treatment failure, Iran.

The Relation of Climatic Factors with Prevalence of Cutaneous Leishmaniasis

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Introduction: Leishmaniasis is a parasitic infectious disease, which is caused by a protozoan called *Leishmania*. It appears in three clinical forms including Cutaneous Leishmaniasis (CL), Mucocutaneous Leishmaniasis (MCL), and Visceral Leishmaniasis (VL). Anthroponotic Cutaneous Leishmaniasis formerly called urban form mostly observed in cities while Zoonotic or rural form mostly seen in villages. Climatic factors have effective influence on the incidence and prevalence of CL. The purpose of this study was to investigate the present status of CL in the city of Mashhad as one of the important centers of the disease and the effects of bioclimatic factors on its prevalence during the past 22 years (1992 – 2014).

Methods: In this epidemiological study, the raw data of confirmed cases with CL were collected from 5 health centers of Mashhad University of Medical Sciences. The climatic data were also obtained from the Meteorological Office and then analyzed using Excel software and Pearson correlation test.

Results: During 20 years (1992–2014), 68958 cases of CL were diagnosed at Mashhad health centers, the highest rate of CL was observed among the patients who had referred to the mentioned health centers during the cold seasons every year. No significant relationship was found between sunny hours and wind speed and CL, but there was a remarkable correlation between precipitation rate, seasonal temperature, and humidity percentage with the prevalence of the disease. The highest rate of CL infection was observed during 2000-2001 with higher prevalence of ACL.

Conclusion: Climatic factors have remarkable influence on the prevalence of ACL in this city. Most of the patients were found to live at the marginal areas of the city with a non-immune population, low municipal services, new constructions, and reservoirs of the disease.

Keywords: Climatic Factors, Prevalence, Cutaneous Leishmaniasis, Mashhad

A Case-Control Study of Relationship between *Toxoplasma gondii* Infection and Schizophrenia by Using Serological and Molecular Methods

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Objective: The occurrence of some infections including *Toxoplasma gondii* during pregnancy or around the time of birth could be increased the risk of schizophrenia. The aim of the study was to evaluate the association between *Toxoplasma* infection and schizophrenia using serological and molecular methods.

Method: From June 2016 to February 2017, 60 inpatients with schizophrenia (case group) among 456 inpatients and 40 healthy volunteers of the psychotic inpatients family (control group) were selected in Ibn-e-Sina and Hejazi hospitals, In Mashhad, Iran. The blood samples were taken for each person and examined by serological and molecular methods.

Results: Overall seroprevalence *T. gondii* antibodies in case and control groups were 55% and 45%, respectively ($P>0.05$). The seroprevalence differences of the infection in case and control groups with regard to age and sex as risk factors were not significant ($p>0.05$). The results of study show that autumn born inpatients seroprevalence of infection was higher than same group in controls, and it was significant ($P<0.05$). The results of nested-PCR indicated that 21% of seropositive case group and 55.5% of seropositive control had *Toxoplasma* DNA in blood samples ($P<0.05$).

Conclusions: It was concluded that *T. gondii* infection could not be considered as a risk factor to schizophrenia, despite the high frequency of *Toxoplasma* infection in the schizophrenic patients. It seems that the differential diagnosis of congenital and acquired toxoplasmosis in the schizophrenic patient is critical for the better understanding association between the *T. gondii* infection and schizophrenia.

Key-words: *Toxoplasma gondii*; Schizophrenia; Serology; PCR

Prevalence and Associated Risk Factors of Soil Transmitted Helminthic Parasites in Iranian Children with Hypereosinophilia

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Background: Eosinophilia may have different causes, from infectious to non-infectious. Here we investigated the prevalence of the intestinal helminthic parasites among children with hypereosinophilia and healthy children from Lorestan province, western, Iran.

Methods: This case-control study was carried out from July 2017 to August 2018 on 224 children (ranging from 2 to 15 years old) including 112 children with peripheral blood eosinophils greater than 1000 per microliter who referring to health centers of Lorestan province, Iran (case group) and 112 healthy children with normal peripheral blood eosinophils. The microscopic examination was performed on the stool samples from each child by means of the direct smear, and formol-ether methods. An applied questionnaire was considered to reach information about the children's demographics data and other variables related to helminthic infections.

Results: Out of 112 children with eosinophilia (case group), helminthic infection were found in 23 (20.5%) children; whereas from 112 health children in control group helminthic infection were found in 12 (10.7%) children; indicating the significant difference ($p < 0.001$) in the prevalence helminthic infection among the children in case demonstrated that a number of risk factors were considerably related to the prevalence intestinal helminthic parasites included gender ($p = 0.013$) and consumption of raw or unwashed vegetables and fruits ($p < 0.001$).

Conclusion: The obtained findings revealed the considerable prevalence of intestinal helminthics parasites among children with hypereosinophilia in Lorestan province, Western, Iran. The results of the present study also suggest that physicians pay more attention to worm infections as an important factor for eosinophilia.

Keywords: Intestinal parasites; hypereosinophilia; formalin-ether

Evaluation of Apoptosis in Splenic Macrophages of Dogs with Visceral Leishmaniasis

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Abstract: Visceral leishmaniasis (VL) is a deadly disease caused by infection with the *Leishmania* parasite. As many as 310 million people are at risk of infection. Leishmaniasis is a fatal disease for the dog if not treated. The treatment can only reduce the importance of crisis, because the parasite stays alive in the different organs. Macrophages are one of the most important cells which have a critical role in the pathogenesis of this disease. The main objective of this study was to evaluate the rate of apoptosis occurrence in splenic macrophages of affected dogs by visceral leishmaniasis.

Materials and Methods: The subject included 12 male stray cross breed dogs affected by visceral leishmaniasis at the age of 5 ± 0.2 and weight of 20 ± 1 kgs and 12 healthy dogs with similar characteristics at the age of 4 ± 0.2 and weight of 25 ± 1.4 kgs. Dogs were purchased from the place where stray animals were kept in Tabriz and Urmia. The spleen of the dogs was microscopically studied using H&E and TUNEL staining. The data collected were analyzed using t-Test. Values Of $p < 0.05$ were considered statistically significant.

Results: The occurrence rate of apoptosis in splenic macrophages of disease dogs (5.2 ± 0.358) was significantly ($p < 0.05$) higher than those of healthy dogs (1.1 ± 0.3163).

Conclusion: The results obtained from this study indicated that lymphoid depletion of splenic tissue in affected dogs by visceral leishmaniasis due to the release of multiple induced cytokines apoptosis occurs.

Keywords: Leishmaniasis, Apoptosis, Macrophage, Spleen, Dog

Pseudo-Malaria (*Haemoproteus* spp.) Infection and *Pseudolynchia canariensis* Infestations in Pigeons in Bandar Torkaman City, Iran

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Haemoproteus columbae widely occurs in pigeon in tropical and subtropical regions. Infection with this genus is sometimes known as pseudo-malaria because of the parasites' similarities with *Plasmodium* species.

The aim of present study was to evaluate the prevalence of *H. columbae* and rate of *Pseudolynchia* infestation in pigeons in Golestan Province, Iran. one hundred pigeons were examined for *Haemoproteus* spp and *Pseudolynchia canariensis* in Golestan Province, Iran between September and November 2018. Firstly, whole bodies of pigeons were inspected and the pigeon louse flies were taken into 70% alcohol. Blood films, fixed in absolute methanol and stained with Giemsa and finally rate of parasitemia calculated. Data were analyzed with SPSS analysis software. Differences expressed as significant at $P < 0.05$.

Mature and immature stages of *H. columbae* gametocytes were found in 89% of blood smears prepared from 100 healthy domestic pigeons. The rates of *H. columbae* infection in female pigeons (49%) were higher compared to those in males (40%). Mean of parasitemia in infected pigeons was 3.39%.

There was 85 (85%) cases of *H. columbae* infection in adolescent birds. In 35 pigeons (35%) *Pseudolynchia canariensis* was observed.

This study show the prevalence and rate of parasitemia in domestic pigeons is high.

Thus the detection of *Haemoproteus* spp. in wild and domestic birds is very significant because of their movement from one source to another and contact with their intermediate host lead to spread pseudo-malaria infection.

Keywords: *Haemoproteus columbae*, *Pseudolynchia canariensis*, Bandar turkaman, Iran

The yeast *Wickerhamomyces anomalus* as an Agent for the Symbiotic Control of Malaria: A Review

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Introduction: Malaria is one of the most common vector-borne infectious diseases threatening millions of people in tropical and subtropical areas, including Africa, Asia and the United States. In the absence of appropriate and effective vaccines, the use of insecticides to reduce the number of mosquitoes and also drug use to kill the parasites are considered as public health programmes. Unfortunately, these chemical and pharmaceutical treatments, because of increased resistance to drugs and insecticides, are not so effective and are considered as a challenge in the present and future. Therefore, new control strategies are necessary to control and suppress malaria. In the last 10 years, the gut microflora of the mosquito has been investigated because it is likely that these microorganisms could interfere with the transmission of the disease by the mosquito. The yeast *Wickerhamomyces anomalus* (known as *Pichia anomala* or *Hansenula anomala*) isolated from the malaria mosquito vector *Anopheles stephensi* attracted wide attention for many years due to its broad biotechnological potential and also production of killer toxins (WaKT) against other microorganisms.

Materials and methods: In the present study, the collection of materials was done by searching the keywords (*Wickerhamomyces anomalus*, *Anopheles stephensi*, *Plasmodium berghei*, Malaria, Symbiotic control, Killer toxin) in the Google, PubMed, SID, Iran Medex databases.

Results: The results of the studies in vitro and in vivo (in the mosquito's body) showed the colonization of *Wickerhamomyces anomalus* in pre-adult and adult stages of mosquitoes by isolation in pure culture. *W. anomalus* strain isolated from *An. stephensi*, WaF17.12, is able to produce a killer toxin (KT) with antimicrobial activity in a non-cellular medium and also in the mosquito's body. The high anti-*plasmodium* effect of this toxin was observed (an inhibitory percentage up to 90%) in *P. berghei* sporogonic stages treated with KT.

Discussion and conclusion: Isolation and detection of the yeast *Wickerhamomyces anomalus* in insects gut and gonads including *Anopheles* mosquitoes open new ways to control and fight vector-borne infectious diseases such as malaria. With reduction or elimination of the ability of mosquitoes to transmit the disease, the delivery of effector molecules to mosquito vector of *Plasmodium* using eukaryotic microorganisms and most importantly, the use of antimicrobial effect of the yeast - the production of killer toxins (KTs) - against human pathogens and parasites such as malaria, researchers hope to a protection or symbiotic control against vector-borne infectious diseases such as malaria.

Keywords: *Wickerhamomyces anomalus*, *Anopheles stephensi*, *Plasmodium berghei*, Malaria, Symbiotic control, Killer toxin

Identification of Gastrointestinal Parasites in Rural Horses in Hamedan Province

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Objective: The horses have been a symbol of bravery and power since ancient days and this taboo is still exists in our society. Equine acts as a main means of transport of goods and economy for poor farmers. This study aimed to determine the prevalence of gastro-intestinal parasites of horse in five Village of Hamedan province.

Methods: Fecal samples for detection of gastrointestinal parasites were collected from 40 working horses (17 males and 23 females) from May to June 2019 from 5 villages in Hamedan, West of Iran .The samples microscopically examined using techniques included: Clayton- laen method of floatation test using saturated sodium chloride solutions and sugar saturated solutions.

Results: Fecal samples of 6 horses (15%) were negative for parasite eggs or oocysts. 57.5% were infected with a single parasite type and 10 (25 %) of horses had multiple infections with two and three parasites. The highest prevalence belonged to *Parascaris* (32.5%). The overall prevalence of intestinal parasites eggs and oocyst in the positive horses were *Parascaris equorum* (32.5%), *Dictyocaulus* 10%, *Dicrocoelium dendriticum* 7.5%, *Eimeria leuckarti* 5%, *Strongyle* 2.5%.

Conclusion: This study suggests that the high rate of infection with gastrointestinal parasites could contribute to low performance and life expectancy of working horses in the region.

Keywords: Horse, Parasite, Hamedan

Genotyping Investigation of *Toxoplasma gondii* Parasites Isolated from Placenta of Human spontaneous aborted Fetuses in Jahrom by RFLP-PCR assay

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Introduction: Congenital Toxoplasmosis is one of the major causes of spontaneous abortion, which is due to the contamination of the fetus with *Toxoplasma gondii*. Different strains of *T. gondii* are found in 3 main genotypes, which are different from each other in the pathogenesis. The aim of this study was to determine the genetic diversity of *T.gondii* parasites isolated from placenta of aborted fetuses spontaneously, in Jahrom by PCR-RFLP method.

Materials and methods: A total of 330 placenta samples were collected from aborted fetuses spontaneously, in Jahrom in Fars province, Iran. DNAs were extracted from the placenta tissues. A Nested-PCR assay was performed to detect *T.gondii* using the 529 bp fragment as a target. Genotyping was done by Nested PCR-RFLP based on GRA6 gene.

Results: The prevalence of *T.gondii* infection among aborted fetuses was found to be 14.5%. The PCR-RFLP method was able to determine the genotype of 9 samples that all belong to Genotype II.

Conclusion: In the present study, a significant frequency of *T.gondii* among the aborted fetuses (14.5%) can indicate the probable role of this parasite in a significant percentage of spontaneous abortion in Jahrom. Although researchers have considered the pathogenicity of I more than II and III genotypes but in most studies on aborted fetal specimens (including the present study), genotype II has been found to be a considerable frequency which may reminder an association between genotype II and abortion.

Keywords: *Toxoplasma gondii*, Aborted fetuses, Genotype, Jahrom

Molecular Study of *Neospora caninum* Infection in Ruminants Aborted Fetuses in Northern Iran

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Background: Numerous studies have been performed about infection of livestock with *Neospora caninum* in Iran that show neosporosis should be considered as a cause of economic and health problem in the livestock industry. The objective of this study was to identify *N. caninum* DNA in brain samples of aborted fetuses of ruminants in Mazandaran province, northern Iran, by nested PCR method.

Material and Methods: In total, 133 aborted fetuses (51 sheep, 78 cattle, and 4 goats) were collected from different stages of gestation in various regions of Mazandaran province from March 2016 to May 2017. DNA was extracted from all brain samples using Phenol Chloroform Isoamyl Alcohol instructions. NC-5 gene was used for the detection of *N. caninum* DNA by the nested-PCR assay.

Results: The detection of *N. caninum* DNA was confirmed by observation of a 227 bp band in 24 out of 133 aborted fetuses (18.1%). The highest prevalence rate of *N. caninum* detected from cattle (20.5%) followed by sheep (15.6%), whereas no positive cases were reported from the goat. The highest prevalence of the infection was observed in Qaemshahr area (23.8%), while the lowest prevalence of the infection observed in Behshahr area (8.6%). The prevalence of infection in the early gestation period (32%) was higher than the middle (15%) and late (3.8%) period.

Conclusions: Results indicate that *N. caninum* infection may be responsible for abortion and economic losses in livestock farming. Therefore, further additional researches for designing control strategies for improving management of ruminant aborted fetuses are necessary.

Keywords: *Neospora caninum*, aborted fetuses, Mazandaran

In Vitro Ovicidal Activity of Nematophagous Fungus *Paecilomyces lilacinus* on the Eggs of Parasitic Helminths

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Introduction: The nematophagous fungi have been suggested as an alternative way to eliminate the zoonotic helminths eggs from the environment. In the present study, we evaluated the ovicidal activity of a fungus, *Paecilomyces lilacinus*, recovered from a compost soil, on the eggs of parasitic helminths under in vitro condition.

Methods: Water suspension of the soil samples collected from different areas of Iran were transferred to 2% water-agar culture media baited with *Rhabditis* sp. larvae. The nematode larvae trapped by fungi were transferred to potato dextrose agar (PDA) media. The sequencing of a beta-tubulin gene identified the nematophagous fungi as *P. lilacinus*. Eggs of three helminth species, *Syphacia obvelata*, *Hymenolepis diminuta* and *Echinococcus granulosus* were exposed to one isolate of the recovered fungi, and the ovicidal activity was monitored for up to 21 days.

Results: Out of 300 samples, only three compost soils, contained the nematophagous fungus *P. lilacinus*. Microscopical examinations revealed invasion of the helminths eggs by the *P. lilacinus*. The eggs of *H. diminuta* were more vulnerable to this fungus invasion while *E. granulosus* eggs were the least affected ones ($P < 0.05$).
Conclusion: *Paecilomyces lilacinus* can grow on fresh feces and attack the eggs of the parasitic helminths. Therefore, a combination of its hyphae with feces or administration of the spores in the animals' food may reduce the helminths eggs in the environment and consequently intervene with the transmission of parasitic helminths in the animal houses.

Keywords: Nematophagous, *Paecilomyces lilacinus*, *Syphacia obvelata*, *Hymenolepis diminuta*, *Echinococcus granulosus*.

Toxocariasis, a Zoonotic Disease, Threatening the Health of Students and Employees of University Complex of Lorestan University of Medical Sciences

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Introduction: the mature parasite of *Toxocara*, which lives and breeds in the small intestine of the dogs and cats as the main hosts, belongs to roundworms of the Ascarididae family. When embryonic eggs enter the digestive tract through the soil, it causes the spread of zoonotic Toxocariasis which has global spread. The goal of this research is to investigate the presence of *Toxocara*'s egg in the vicinity of the university complex of the Lorestan University of Medical Science which is considered as a threat to the students and staff.

Materials and methods: The university complex is located in a low foothill region in 5 km from Khorramabad city, the center of Lorestan province. Twenty-two soil samples were collected from various locations around the complex, such as streets, street edges, pavements, and lawns, and then in order to detect *Toxocara* parasites, they were filtered using metal filters based on O'Lorcain method.

Results: Six samples (27.3%) of the soil samples were contaminated with *Toxocara* eggs and the samples of the park lawns were found most contaminated.

Discussion: The abundance of dogs and cats in the geographical environment of the university campus and the relatively high *Toxocara* parasite in peripheral soils is considered a threat to employees of the university who works there, as the staff and students who spend their free time in these spaces despite serving meals in nearby parks increases the risks of Toxocariasis infection. Therefore, it is suggested that employees who involved with the soil be screened for seroepidemiology for more consideration, in case of high prevalence.

Keywords: *Toxocara*, Soil, Lorestan, infection risk, university campus

The Prevalence of Intestinal Parasites among Pet Dogs in Dalahoo and Songhor counties, located in Kermanshah Province, Iran

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Introduction: The dog, as one of the animals forming close relationships with humans, are considered to be a major transmitter of zoonoses, where the transmission frequently occurs via the eggs and larvae of parasitic worms living in the soil. The purpose of this study is to investigate the prevalence of intestinal parasites among the pet dogs in two counties located in Kermanshah, Iran.

Methodology: 54 fecal specimens of pet dogs were collected in the mornings during the winter 2016, and analyzed using direct saline and Lugol's iodine wet mount and Formalin-Ether sediment concentration, magnified to the power of 10x and 40x, by means of an optical microscope.

Results: 28% of the fecal specimens were infected with single-celled parasites including *Giardia canis* and *Entamoeba coli*, and parasitic worms including *Ancylostoma caninum* and various species of *Hymenolepis*, containing 3.75% of each, and 13% of the pet dogs or 46.7% of parasites including *Toxocara canis* worm. Meanwhile, 73.3% of the infections were caused by parasitic worms, whereas the single-celled parasites built up only 26% of the infections.

Discussion: Pet dogs and farm dogs living in the abovementioned counties were infected almost at the same rate, but compared to the pet dogs of other provinces, the infection rate was higher; however, when compared to the stray dogs which feed on poor-quality foods, while being deprived of veterinary care or antiparasitics, the infection rate is considerably lower. It is recommended that the local veterinary offices regard the findings of these studies as tangible evidence and take proper action for the treatment and prevention of these infections by special medications.

Keywords: Intestinal parasites, pet dogs, Dalahoo, Songhor, *Toxocara*, Kermanshah

Parasitic Contamination of Drinking Water wells in Rumeshgan, Lorestan Province

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Healthy drinking water is a source of life and comes from many sources, most notably groundwater which is infectious in both chemical and biological forms. One of the biological factors is parasitic infections, especially protozoa, causing deaths

or erosion of the physical and mental power of infected individuals annually. The purpose of this study is to investigate the prevalence of parasites in drinking water wells in the city of Rumeshgan. Cross-sectional study is designed and 132 samples of 2.5 liters of drinking water from wells in the city of Rumeshgan are collected haphazardly. The parasites in each of the samples are concentrated on the surface of the filter paper using a suction pump, then, by washing the filter paper and centrifuging the solution of the rinse, the condensed parasites in the sediment are stained with Logel and Zill- Nelson methods, using an optical microscope the survey was conducted. The parasites in each of the samples are concentrated on the surface of the filter paper using a suction pump. Then, by washing the filter paper and centrifuging the solution of the washer, the condensed parasites are stained in the sediments by Logel and Zill-Nelson methods and surveyed by optical microscope. Data analysis is performed using descriptive and analytical statistics. In current study, 14.4% of drinking water wells are infected with pathogenic parasitic protozoa and the most common parasitic infection is cryptosporidium has that appropriated of 9.8% of water wells. Parasitic contamination with the well water depth of [OR = 5.2 95% CI (2-6.8)] and the distance of water well to the sewage well [OR = 3.5 95% CI (1-3.5)] had a significant relationship. SIG <0.05

Interpretation: Contamination of 14.4% of drinking water from wells to pathogens causes at least annual referring of Consumer population to the physician and to the imposition of economic and health damage especial in hot seasons.

Statistical results, along with other reasons, indicate that drinking water pollution is due to leakage of sewage sludge, which is suggested to provide water for plumbing or controlling for people. Otherwise, the epidemic of water borne diseases is not unexpected.

Keywords: Well Drinking Water, Sewage Well, Parasitic Contamination, Water Well Distance to Wastewater, Rumeshgan County

Anti-*Trichomonas vaginalis* Activities and Apoptotic Effects of Some Iranian Medicinal Plants

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Trichomonas (T.) vaginalis a protozoan parasite that causes Trichomoniasis is one of the most widespread sexually transmitted diseases. Aim of the present study is to evaluate the in vitro antitrichomonal properties of plant extracts of *Quercus (Q.) infectoria*, *Pistacia (P.) khinjuk*, and *Satureja (S.) khuzestanica* that are ethno-medicinally used in Iran against *T. vaginalis* trophozoites. In this study, the in vitro anti-*T. vaginalis* activities of the *Q. infectoria*, *P. khinjuk*, and *S. khuzestanica* extracts against *T. vaginalis* clinical isolates were assessed by Trypan Blue exclusion assay. The effect of the extracts on induced apoptosis in *T. vaginalis* trophozoites was evaluated using the fluorescein isothiocyanate (FITC) Annexin V staining kit. The *Q. infectoria* methanolic extract was significantly ($P<0.001$) more effective than the other tested extracts. It demonstrated lower IC50 values for trophozoite of *T. vaginalis*. *Q. infectoria* methanolic extract exhibited significantly ($P<0.001$) a higher rate of apoptosis on *T. vaginalis* trophozoite than other tested extracts and control group. Results of the study revealed that *Q. infectoria* extract can be considered as a suitable choice for medical studies to treat trichomoniasis. However, additional clinical studies are necessary to evaluate accurate biological effects of this plant on volunteer human subjects.

Epidemiology of Pathogenic Parasite *Histomonas meleagridis* in Poultry in Lorestan Province, Western Iran

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Histomonas meleagridis is a flagellate protozoan parasite living in the cecum of birds digestive system and is the causative agent of histomoniasis. In this study 110 poultry fresh stool samples were assessed in order to detect *H. meleagridis*, and egg or adult worm of *Heterakis gallinarum* in Lorestan province, Iran. The results showed that the prevalence of *H. meleagridis* was 31%. Also, 19.5% of infected poultry had a watery stool. The eggs or adult worms of *H. gallinarum* were not seen in any of the samples. The results showed the high prevalence of this parasite, and the factors such as temporary hosts, susceptible hosts, and cloacal transmission with the involvement of water containing the parasite are the effective risk factors in prevalence. It is better to considered *H. meleagridis* as waterborne parasites, so further epidemiological studies on other birds are suggested to determine the parasite pathogenic strains using molecular methods.

Keywords: Parasite , Transmission, Prevalence, Epidemiology , Cloaca.

In Vitro and in Vivo Antileishmanial Activities of *Quercusinfectoria* Olivier Extract

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Background: During recent decades, studies have demonstrated that a number of plant derived compounds may act as new therapeutic tools against leishmaniasis. This study was evaluated the antileishmanial, and cytotoxic activities of *Quercusinfectoria* Olivier (oak) extract.

Methods: This extract (0–80 g/mL) was evaluated in vitro against promastigote and intracellular amastigote forms of *Leishmania major* (MRHO/IR/75/ER) using MTT assay and in a macro-phage model, respectively. Then oak extract was tested on CL in infected male BALB/c mice with *L. major* in order to evaluate the antileishmanial activity topically. Moreover, cytotoxicity effects of oak in murine macrophage cells were tested by MTT assay.

Results: The findings revealed that oak significantly ($P < 0.05$) inhibited the growth rate of promastigote of (IC₅₀ 12.65mg/mL) and amastigotes (IC₅₀ 10.31mg/ mL) as a dose-dependent response. In the in vivo assay, after 4 weeks of treatment, 91.6, 66.66, and 50% recovery was observed in the infected mice treated with 20, 10, and 5 mg/kg of oak extract, respectively. After treatment of the infected mice with the concentration of 10 and 20 mg/kg of oak, the mean diameter of lesions, parasite load and mean number of parasites was significantly ($P < 0.05$) reduced. Selectivity index of greater than 10 for oak revealed that oak extract had no cytotoxic effects on macrophage cells.

Conclusion: To conclude, the present study showed potent antileishmanial and activity of oak extract; whereas this plant had no toxic effect on mammalian cells.

Keywords: antileishmanial; *Leishmania major*; cytotoxic activities; MTT assay

Leishmaniasis and Vaccine

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Introduction: Leishmaniasis or cutaneous leishmaniasis is an inflammation of the *Leishmania* parasite. *Leishmania* is a single-cell parasitic virus living in the human body or animal and mosquitoes carrying the disease and it is one of the most common parasitic diseases in the tropical and subtropical regions of the world.

Method: In this systematic review, after reviewing the Sid information source and the google scholar search engine, and using library searches, the Leishmaniasis and vaccine keywords were achieved. The criteria for entry of articles include from 2011 to 2019 the full text of articles was accessible.

Result: Based on current treatment studies, the types of Leishmaniasis are based on chemical drugs, increased resistance of the disease agent to drug, the increased resistance of carriers to insecticides, the lack of proper and sufficient fighting with reservoir animals, insufficient control measures, population increase and migration and follow-up services. Due to sensory and non-sensory signs, the role of effective vaccine against Leishmaniasis has a significant effect on infected spots in the spread and increase of Leishmaniasis. The following should have the following characteristics: The vaccine should be able to provide long-term immunity to the vaccine. Clinical lesions of Leishmaniasis should be immunized. The vaccine can be used to provide long-term immunity. It should be produced in mass-quantity and selling price should be proper and have no secondary pollution.

Conclusion: The success of the *Leishmania* vaccine has clearly shown the need for research and investment in the production of a suitable vaccine against *Leishmania*. It is necessary to prevent development of disease, more researches must be carried out.

Keywords: Vaccine, Leishmaniasis

Comparison of the RE-529 sequence and B1 Gene for *Toxoplasma gondii* Detection in Blood Samples of the at Risk Seropositive Cases Using uracil DNA glycosylase Supplemented loop-mediated Isothermal Amplification (UDG-LAMP) assay

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Introduction: Diagnosis of Toxoplasmosis is an important issue, especially in at-risk patients. The molecular methods showed a promising future for such diagnosis, however, the method itself and the target sequence to be detected is an important part of accurate detection of the infection. The aim of the present study was to compare the B1 gene and the RE-529 sequence for *Toxoplasma gondii* detection in blood samples of the at risk seropositive cases using uracil DNA glycosylase supplemented loop-mediated isothermal amplification (UDG-LAMP) assay.

Materials and methods: In the present study, the two most used sequences (RE-529 and B1) used and compared for accurate and reliable detection of *Toxoplasma gondii* in blood samples using UDG-LAMP assay. The detection limit, accuracy and reliability of UDG-LAMP for the parasite's DNA was also studied. For this purpose, 110 *T. gondii* seropositive at-risk individuals (pregnant women and immunocompromised patients) and 110 seronegative controls were enrolled and studied.

Results: Among 110 studied cases, 39 (35.45%) and 36 (32.7%) were positive for *T. gondii* DNA with the RE-LAMP and B1-LAMP, respectively. The seronegative cases remained negative for *T. gondii* DNA with the studied genes. The detection limit of the UDG-LAMP for both DNA targets was 0.16 tachyzoite's DNA per reaction tube.

Conclusion: Based on the results, RE-529 sequence has a better detection rate compared to the B1 gene for Toxoplasmosis among at risk people. UDG-LAMP is a highly sensitive, accurate and reliable method with no false-positive results for diagnosis of *T. gondii* infection in blood specimens.

Keywords: loop-mediated isothermal amplification, *Toxoplasma gondii*, B1 gene, RE 529

Prevalence of Intestinal Protozoa among Referred Patients to 29 Bahman Hospital in Tabriz, 2018-2019

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Background & Objectives: Intestinal protozoan infections are an important health problem in the world, especially in developing countries. Considering the epidemiological importance of parasitic diseases, in different areas and populations, current study aimed to determine the prevalence of intestinal protozoan infections in patients admitted to the laboratory of 29 Bahman hospital in Tabriz.

Methods: This cross-sectional study was performed in March 2017 to March 2018. Stool samples were collected from 2024 patients who were referred to the laboratory of 29 Bahman hospital. Stool specimens were examined for the presence of trophozoites, cysts, and oocysts using direct wet mount.

Results: Stools of 205 (12.3 %) patients were positive for protozoan parasites. Out of 205 individuals, 107 (42.8 %) were female and 98 (39.2%) were male. The highest infection rate (49.6%) was found in <14 yr age group. Amongst the positive sample, 42.2% were infected with *Entamoeba* spp, 37.7% with *Blastocystis hominis*, 13.4% with *Giardia lamblia*, 5.8% with *Endolimax nana* and 0.9% were infected with *Trichomonas hominis*.

Discussion & Conclusion: The result of this study showed the highest prevalence of intestinal protozoa was related to *Entamoeba* spp. Despite the improvement of the public health level, parasitic infections are still considered as one of the health problems in Iran. Therefore, health education and raising the level of health culture in the region are among the ways to reduce parasitic infections in different regions.

Keywords: Intestinal protozoa, Prevalence, Tabriz

The First Report of *Lyperosomum corvi* (Digenea: Dicrocoeliidae) Infection from Eurasian Magpie (*Pica pica*) in Isfahan Region, Iran; a Case Report

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In this investigation, the trematode *Lyperosomum corvi* (Digenea: Dicrocoeliidae; Yamaguti, 1939) was recovered from the liver and bile ducts of Eurasian magpie (*Pica pica*; family Corvidae) in Isfahan region, central Iran, which were studied and identified using valid taxonomic keys according to morphological and structural characteristics. The trematode's characteristics were determined based on the three recovered mature worms which were the same as the first report of *L. corvi* from *Corvus corone* in 1997 in Isfahan. The trematode's body was elongate and flattened dorsoventrally. Its size was 0.8 - 5.5 mm long by 0.86 to 0.6 mm wide, usually wider at acetabulum. The tegument was clear and delicate, thus the internal organs of the worms were easily visible. Oral sucker had a ventral position, muscular and round 0.37-0.331 mm in diameter located at the anterior end, the acetabulum has seen very muscular 0.999 to 0.742 mm in diameter. Prepharynx absent and the pharynx was muscular. Excretory pore was terminally located. The common genital pore and cirrus sac are located below the throat and above the ventral sucker. The ovary was round and smooth, 0.330 to 0.020 mm in size, situated posterior to the testes. The yolk glands rowed on both sides, continued to $\frac{3}{4}$ of the body lengths. Uterus greatly convoluted, and contains 0.024 to 0.035 mm operculated eggs representing a *Dicrocoelium dendriticum* egg, filling most of all the space between the testes and posterior end of the worm. This is the first report of *L. corvi* in the Eurasian magpie and maybe a new possible host for the trematode in Isfahan region, central Iran. Presence of contamination in a number of crows indicates the establishment of the life cycle of this worm in the region.

Keywords: *Lyperosomum corvi*, Eurasian magpie, crow, Isfahan, Iran

G1 Genotype is Predominant Strain of Cystic Echinococcosis in Lorestan Province

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Introduction: Regarding hydatid cyst is as a human and animal public health problem in Iran including the West of Iran, this research was performed in order to genotypes of *E. granulosus* infecting humans and sheeps in Lorestan province.

Materials and methods: Totally, 18 hydatid cysts of sheep and 20 formalin-fixed paraffin-embedded (FFPE) tissue samples from patients were collected. Protoscoleces or germinal layers were collected from cysts of sheeps, and DNA was extracted. Also DNA extraction was done on FFPE tissue samples. The extracted DNA was used in PCR using specific primers of mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene. Then genotyping was performed by sequencing and analyzing.

Results: In sequencing analysis, all of sheep isolates and FFPE tissue samples belonged to genotype G1 (sheep strain).

Conclusion: The obtained findings demonstrated that the common sheep strain/G1 is predominant genotype in Lorestan province, which is indicating the sheep-doge cycle in this area. Further studies with more sample size should be conducted to be sure of the sheep strain G1 that is predominant strain in this area.

Keywords: Hydatid cyst; human; sheep; *Echinococcus granulosus*

Assessment of *Toxoplasma gondii* Antigens as Diagnostic Markers for Human Acute and Chronic Toxoplasmosis

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Background: *Toxoplasma gondii* is an omnipresent protozoan parasite that infects humans and animals. Globally, an estimated one in three people carry this infection. Toxoplasmosis has two acute and chronic phases connected with IgM and IgG antibodies, respectively. Detection of *Toxoplasma* infection and differentiation between the acute and chronic phases are mainly based on immunoserological tests. So, this study aims to assess *T. gondii* RH strain antigens using the Western blotting to differentiate between acute and chronic infections.

Methods: Nearly, 5×10^6 tachyzoites were injected into the peritoneal cavity of 50 BALB/c mice. The peritoneal fluids of infected mice were gathered after 4 days. Following sonication, the protein concentration was determined by the Lowry method. Subsequently, the molecular weight of fractions was discerned by SDS-PAGE. Afterwards, 70 serum samples were investigated by Western blotting.

Results: Five proteins of approximately 12, 28, 41, 72, and 130 kDa were recognized by IgG antibody present in the sera from patients with chronic Toxoplasmosis. Antigens of 12, 28, 41, and 130 kDa were recognized in all patients implying the sensitivity and specificity of 100% for the test. Two proteins with 10 and 41 kDa were recognized by IgM antibody present in the sera from patients with acute Toxoplasmosis. Antigens of 10 and 41 kDa were recognized in all patients implying the sensitivity and specificity of 100%.

Conclusion: Overall, it can be concluded that the Western blot could be used as a diagnostic tool to characterize the acute and chronic phases of *T. gondii* infection.

Keywords: *T. gondii*, Toxoplasmosis, Acute, Chronic, western blot, Iran

Detection of *Toxoplasma gondii* by Native and Commercial ELISA in Ahvaz, Iran: A comparative Study

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Background: Toxoplasmosis is a parasitic disease caused by the intracellular protozoan parasite *Toxoplasma gondii*, which can infect humans and animals. Currently, routine diagnosis of Toxoplasmosis relies mainly on the use of various serological tests to detect specific antibodies in infected patients. Thus, in this study, a native ELISA kit was designed utilizing *T. Gondii* tachyzoites from the RH strain, and its sensitivity and specificity were contrasted to those of commercial kits.

Methods: *T. gondii* RH strain tachyzoites were injected into the peritoneal cavities of 50 BALB/c mice. After 4 days, tachyzoites were harvested. Subsequently, the concentration of protein was calculated by the Lowry method. The optimum dilutions were determined by checkerboard titration of antigen, serum and conjugate. ELISA was executed based on these dilutions, and its sensitivity was determined using 200 serum samples. Furthermore, the specificity of the assay was assessed using 40 serum samples from patients with tuberculosis, Leukemia, and hydatid cyst.

Results: Native ELISA was employed to check 100 serum samples including anti-*T. gondii* IgG, with a sensitivity of 98% (commercial kits: 100%). Other 100 serum samples containing anti-*T.gondii* IgM were also tested, with a sensitivity of 99% (commercial kits: 100%). As 40 serum samples from patients with leukemia, hydatid cyst or tuberculosis were examined using anti-*T. gondii* IgG, the specificity was exactly alike to commercial kits.

Conclusions: Indigenous soluble crude antigens of *T. gondii* from the peritoneal cavity of mice may be one of the main favorable antigens for detection of human Toxoplasmosis in conventional assessment.

Keywords: *Toxoplasma gondii*, RH Strain, Native ELISA, Commercial Kits, Iran

Epidemiology of *Taenia saginata* taeniosis/cysticercosis: A Systematic Review of the Distribution in Iran

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Background: The zoonotic parasite *Taenia saginata* transmits between humans, the definitive host (causing taeniosis), and bovines as the intermediate host (causing cysticercosis). An overview of the extent of human *T. saginata* infection and bovine cysticercosis is lacking. This review aims to summarize the current epidemiology of *Taenia saginata* taeniosis/cysticercosis in Iran among humans and animals.

Methods: A systematic review was conducted, that gathered published and grey literature, and official data concerning *T. saginata* taeniosis and bovine cysticercosis in Iran using a number of appropriate key words published between 1967 and 2018.

Results: Data for human taeniosis and bovine cysticercosis were found. Human taeniosis and bovine cysticercosis prevalence varied. Ten (27%) reported on human taeniosis and 15 (40.5%) on cysticercosis. *T. saginata* was implicated in all human taeniosis cases. All *Taenia* spp. cysticercosis cases were reported among domesticated pigs and wild animals. Nevertheless, most of these studies were carried out in northern Iran.

Conclusions: This review found *T. saginata* to be the most prevalent and of greater economic and public health significance in Iran. The public health burden of *T. saginata* is assumed to be small as the parasite is of low pathogenicity to humans. However, this review indicates that infection continues to be widespread and this may result in a large economic burden, due to the resources utilized in meat inspection and condemnation or processing with subsequent downgrading of infected carcasses.

Keyword: *Taenia saginata*, Cestode, Beef tapeworm, Bovine cysticercosis, Taeniosis, Iran

Molecular characterization and prevalence of *Cryptosporidium* spp. in cattle in North Khorasan province, Iran

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Introduction and Aim: *Cryptosporidium* is one of the most important intestinal protozoan pathogens with widespread in young animals and in humans and in other hosts, causing substantial economic losses and public health concern. The prevalence of *Cryptosporidium* species in animals and human in North Khorasan province and zoonotic importance is not well documented. This study aimed to identify *Cryptosporidium* spp. infecting cattle.

Methods: in this study to detect molecular identification about *Cryptosporidium* infection in cattle, both modified Ziehl-Neelsen (MZN) staining and PCR-RFLP were used. The fecal specimens were collected from 160 dairy cattle (≤ 6 and > 6 months age), in 3 industrial cattle farms in North Khorasan province, Iran. *Cryptosporidium* sp. oocysts was determined by modified cold Ziehl-Neelsen's staining. Positive fecal specimens were tested by PCR-RFLP according to amplification of 18S rRNA gene using SspI, VspI and DdeI restriction enzymes and DNA sequencing.

Results: The results of microscopic studies revealed 10/160 suspected samples. Nested PCR amplification confirmed 9/10 of microscopic-positive samples. The RFLP analysis of PCR products from each positive sample with restriction enzymes SspI, VspI and DdeI; these results suggest that these PCR products belonged to both *Cryptosporidium bovis* and *Cryptosporidium andersoni*. It seem that *C. andersoni* and *C. bovis* have role of unknown infection agents in North Khorasan province, Iran.

Conclusion: MZN staining procedure is reliable for the routine diagnosis of *Cryptosporidium* and nested PCR targeting 18S rRNA gene are reliable and useful in epidemiological studies of this parasite.

Keywords: *Cryptosporidium* spp.; Cattle; PCR-RFLP; North Khorasan; Iran

Transforming growth factor β 1 expression in liver fibrosis associated with cystic Echinococcosis

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Background and aims: Hepatic fibrosis is a dynamic process that occurs in response to chronic liver diseases resulted by various factors, such as chronic infections, autoimmune reactions, allergic responses, toxins, radiation, and infectious agents. Among the infectious agents, multicellular parasites such as *Echinococcus granulosus* cause chronic inflammation and the development of liver fibrosis. The aim of this study was to assess the transforming growth factor β 1 (TGF- β 1) expression in fibrotic and healthy hepatic tissue of Cystic Echinococcosis (CE) patients.

Materials and Methods: we investigated fibrotic and healthy liver tissue of 25 CE patients.

In order to assess the pathological morphology of fibrotic and healthy liver tissue including deposition of fat, inflammation, and congestion of RBCs with Hematoxylin and Eosin (H&E) staining and for collagen deposition and fibrosis with Masson's Trichrome (MT) staining were used to investigate the development of hepatic fibrosis. TGF- β 1 expression was evaluated by Immunohistochemistry (IHC) staining and Quantitative real-time PCR analysis (qRT-PCR) in fibrotic and healthy liver tissue.

Results: The results of H&E and MT staining indicated the quantities of inflammation, congestion of RBCs, collagen fibers and fibrosis were significantly increased in the fibrotic tissues, but deposition of fat was decreased in the fibrotic tissue. A significant mRNA level of TGF- β 1 was found up-regulated in the fibrotic tissue comparison with healthy tissue. The obtained results of IHC staining revealed significantly increased amount of TGF- β 1⁺ cells in the fibrotic tissues compared the healthy tissues.

Conclusion: According to current study, TGF- β 1 contribute to the development of liver fibrosis in cystic Echinococcosis. Understanding the expression of TGF- β 1 may facilitate clarification of molecular mechanisms of liver fibrosis and contribute to the development of novel therapies.

Keywords: Liver fibrosis, Cystic Echinococcosis, TGF- β 1, Immunohistochemistry, real-time PCR

Sero-Epidemiology of Human Hydatidosis and Prevalence of Hydatid Cyst in the Slaughtered Livestock in SarpolZahab, Kermanshah, Iran in 2018

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Background and objectives: Hydatid cyst is one of the most common parasitic diseases of humans and livestock, which is caused by the larval stage of the *Echinococcus granulosus*. The disease is present in all parts of the world, including Iran, and it is an important concern for public health and economics. The aim of this study was to investigate the prevalence of hydatid cyst in the slaughtered livestock in Sarpol-e Zahab and sero-prevalence of human hydatidosis in this city.

Marital & method: A total of 1000 cattle, 1000 sheep and 1000 goats' viscera examined for hydatid cysts in the slaughterhouse. Also, 736 blood samples were collected from people referring to the health centers' Sarpol-e Zahab city. Demographic data of participants including age, sex, occupation, history of contact with the dog and area of living were collected by questionnaires. Sera samples were examined by ELISA method after transfer to the laboratory. Data were analyzed using SPSS software and the chi-square test.

Results: Anti-*E. granulosus* IgG was detected in 8 sera samples (1.1%) out of 736 sera, including 5(62.5%) males and 3(37.5%) females. There was no significant relationship between positive sera and area of living, educational level, gender and contact history with the dogs. The prevalence of hydatid cysts in livestock was 261 out of 3000 samples (8.7%). The rate of hydatid cysts in the in sheep, cattle, and goats were 188 (18.8%), 45(4.5%), and 28(2.8%) respectively. There was a significant difference between the infection rate in sheep with goat and cattle ($P < 0.05$). The prevalence of hydatid cyst in female animals was 53.6% and in the males was 4.7%, which was statistically significant ($P < 0.05$). The prevalence of hydatid cyst in the liver of sheep, cattle, and goats was 84.04%, 86.7% and 89.3% of infected organs, respectively.

Conclusion: The present study shows the lower prevalence of human hydatidosis compared to those reported in other parts of the country. Therefore, it seems that health measures and raising awareness of people play a major role in reducing the human hydatid cyst. On the other hand, the prevalence of hydatid cyst in the slaughtered livestock is relatively high in this region. Therefore, preventing stray dogs access to offal seems is necessary for controlling the hydatid cyst.

Keywords: Hydatid cyst, Sero-epidemiology, ELISA, Sarpol-eZahab, Iran

***Eimeria* Species in Referred Pet Rabbits and Rodents in a Small Animal Hospital**

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Background and Objectives: Coccidiosis is a common disease in young animals, especially in overcrowded enclosures and poor hygienic conditions. Considering the escalating popularity of rabbits and rodents as pet in recent years in our country, prevalence of different parasites such as *Eimeria* has not been studied in rabbits and rodents. The aim of this study was to determine the frequency and identify the *Eimeria* species in referred rabbits and domestic rodents.

Materials and Methods: For a one year period, from August 2017 to August 2018, stool samples from rabbits and rodents referred to a Small Animal Hospital were collected. The samples were initially examined with wet mount, afterwards flotation concentration was performed for detection of eggs and oocysts. Samples containing oocysts were stored in Potassium Di-Chromate for sporulation, subsequently inspected for species identification based on morphological characteristics.

Findings: Totally the 245 investigated fecal samples belonged to 189 rabbits, 28 guinea pigs, 11 squirrels and 17 hamsters, of which 212 samples (86/5%) had no detectable fecal parasites. *Eimeria* oocysts were found in 27 samples of rabbits, and other hosts were free of coccidian infestation. *E. media*, *E. perforans*, *E. irresidua*, *E. exigua*, *E. piriformis*, *E. coecicola*, *E. magna* and *E. stiedai* were identified.

Conclusion: Despite the low level of infection in this study, clinical liver coccidiosis and the variety of detected *Eimeria* species similar to other studies on wild and laboratory rabbits, non-zoonotic parasites should not be overlooked as an animal welfare issue. Simple fecal examinations in veterinary clinics may be used for detection of possible infections and appropriate treatment and prevention measures may be advised.

Keywords: Rabbits, Rodents, *Eimeria*, Exotic Pets

Prevalence and Economic Impact of Cystic Echinococcosis and Liver Flukes Infections in Slaughtered Sheep and Goat in North-Central Iran: A Single Center's Experience from 2008 to 2018

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Intouduction: Cystic Echinococcosis (CE) and liver flukes infections as important zoonotic infections impose a large socioeconomic impact on societies. As an endemic region for these infections, slaughterhouse inspections should be more considered in Iran. This study aimed to analyze the 11-year record of offal condemnation due to CE, fascioliasis, dicrocoeliasis infections in sheep and goat and its economic impact at Alborz slaughterhouse, north-central Iran.

Methodology: The prevalence rate was calculated as the infected organs (as nominator) divided by the slaughtered cases (as denominator) in each year and month. The Annual Percent Changes (APC) was used to determine trends of parasitic diseases over time. The relationship between metrological indexes and the prevalence of parasitic diseases was determined by the linear regression model. Statistical analyses were done using STATA software 14.

Results: The overall prevalence rate of fascioliasis, dicrocoeliasis, and CE was 0.95%, 2.17%, and 12.74%, respectively. There was a declining trend in the prevalence of fascioliasis and dicrocoeliasis, whereas, the prevalence of CE increased from 7.57% in 2008 to 9.53% in 2018, representing an annual change of +2.0%. The direct economic impact was estimated at US\$ 1,670,977 and US\$ 25,148 for liver and lung, respectively.

Discussion and conclusion: The number of condemned organs due to these infections is noticeable in Alborz Province, north central, Iran. The high economic impact of these infections showed the necessity of implementation a continuously infected animals trace-back and disease control in the site of infection.

Keyword: Cystic Echinococcosis, fascioliasis, dicrocoeliasis, economic impact, Iran.

Anisakidae larvae in edible fish and investigation of its post-mortem migration into fish flesh: A study in Southern Iran

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Introduction: Anisakidosis is a worldwide fish borne ascaroid zoonotic parasitic disease causing allergies in human by accidental ingestion of uncooked or undercooked fish. The aim of the study was to determine the prevalence and intensity of Anisakidae larvae and investigation of the possibility of post-mortem migration of larvae into the flesh [which could be a source of by food-borne allergen due to anisakids larvae] of the fish in a number of commercially important fish in Iran.

Methodology: Different fishes including *Psettdodes erumei* [KAFSHAK] (n=52), *Surida tumbil* [HASOUN] (n=42) were collected from Bushehr shores in the Persian Gulf, between spring 2015 to winter 2016. Samples were taken from body cavity and filet of fishes. Parasitological examinations were performed for anisakids by routine autopsy methods. Pepsin assay was also performed for investigation of the worm burden or density by counting the number of parasites per kg of fish.

A total of three pieces fish filets from three fish from each fish species were also investigated by histopathological examinations.

Results: The prevalence of ascaroid contamination for (*Surida tumbil*) and (*Psettdodes erumei*) were 42.85% and 15.09%, respectively. Morphological and morphometric measurements showed the presence of *Anisakis* larva type I and *Hysterothylacium* spp in the fishes. From 336 obtained ascaroids larvae in *Psettdodes erumei*, 239 (71.13%) were *Hysterothylacium* spp. and 65 (19.34%) were *Anisakis* type I while from 308 obtained ascaroids larvae from *Surida tumbil* 266 (73.37%) were *Hysterothylacium* spp. and 10 (3.24) *Anisakis* type I. Pepsin assay showed presence of 10 (2.97%) *Anisakis* type I and 10 (2.97%) *Hysterothylacium* spp with the density of 6.25 per kg for both larval family in *Psettdodes erumei* fish filets; while in *Surida tumbil* 3 (0.97%) and *Anisakis* type I and 11 (3.57%), *Hysterothylacium* spp with density of 0.83/kg and 2.77/kg respectively. The range of larval intensity infection was measured to be 1-35 in both fishes. Histopathological examinations of *Psettdodes erumei* and *Surida tumbil* fishes showed the sections of nematodes resembled to *Anisakis* type I and *Hysterothylacium* spp in both fishes.

Discussion and conclusion: These results indicated that anisakidosis which have been reported as food-born allergens in all over the world are common in this region. They could be regarded as a health hazard for people in the region under study. New standard and sensitive method should be developed for detection of these larvae in fish filets to reduce the risk of human contaminations.

Keywords: Anisakids Larvae, Allergen, *Psettdodes erumei*, *Surida tumbil*, Pepsin assay, fillet infections Iran

Prevalence of hydatid cysts in slaughtered animals in Hamedan City Western of Iran

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Background and Objectives: Echinococcosis/Hydatidosis is a public health concern and is endemic in many parts of the world including in Iran which an important medical and veterinary problem, Domestic intermediate hosts are a major reservoir for the disease in humans. Hydatidosis is a cosmopolitan disease and occurs in all continents but high prevalence is found in some parts of Eurasia, Africa, Australia, and South America. The aim of this study was to determine the prevalence of hydatid cysts in slaughtered animals in Hamedan City.

Methods: In this cross-sectional study performed from 21Mar²⁰¹⁸ to 19Mar²⁰¹⁹, a total of 83160 animals (11072 cow and 72088 sheep) were inspected macroscopically for hydatid cyst.

Results: Prevalence rate of hydatid cyst in liver of cow and sheep was 810(7/3) and 11318(15/7).

Moreover infected lungs of cow and sheep was 1608(14/5) and 1816(25/1) respectively.

Most condemnation cases were seen in lung of sheep (25/1). It appears that sheep are the most important intermediate hosts for *Echinococcus granulosus* (*E. granulosus*) in this area.

Discussion and Conclusion: The results of this study indicates high prevalence hydatid cyst in slaughtered animals in Hamedan City and the potential role of stray dogs in the transmission of zoonotic parasites must be considered by the public health authorities and veterinarians.

Rahmati and colleagues (2015) in a study in Hamedan city reported out of the examined 103 stray dogs 37/9% of examined stray dogs were infected with *E. granulosus*. Therefore, control the stray dog population, to avoid contamination of environment, water supplies and food with dogs' feces was recommended. It is essential that public health authorities and veterinarians develop control strategies for decreasing the human infection, which requires more inclusive and comprehensive sanitary and control measures due to this parasite's life cycle and transmission.

Keywords: Hydatid cysts, slaughtered animals, prevalence

Isolation and Molecular Identification of Free-living Amoeba from Agricultural Water Canal in Northwest of Iran

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Background: Free-living amoeba (FLA) are widely distributed in different environmental sources. The most genera of the amoeba are *Acanthamoeba*, *Naegleria* and *Vermamoeba*. The most common consequences of the infections in immune-deficient and immunocompetent persons are amoebic encephalitis and keratitis. The aim of the present study was to investigate the presence of *Acanthamoeba* spp. and *Naegleria* spp., isolated from the main agricultural water canal in Qazvin.

Methods: Totally, 120 water specimens were collected and later the specimens were cultured and cloned to identify positive samples. PCR amplification and sequencing were carried out to identify the isolated species as well as the genotypes of amoeba.

Results: According to morphological surveys, 41.7% (50/120) of water specimens were positive for FLA. Molecular analysis revealed that 68.6% and 31.4% of *Acanthamoeba* specimens were identified as T3 and T4 *Acanthamoeba* genotypes, respectively. Also two other species of *Naegleria* named as *N. lovaniensis* (57.1%) and *Naegleria* sp (42.8%) were identified. The results of pathogenicity assays demonstrated that 38.5% of T3 and 61.5% of T4 genotypes of *Acanthamoeba* were highly pathogenic parasites.

Conclusion: This study showed that the water flowing in the agricultural canal of the area is contaminated with potential pathogenic FLA, therefore, it is recommended that more attention to be paid towards proper treatment of water sources to prevent possible risk of the disease.

Keywords: *Acanthamoeba*, *Naegleria* spp, genotype, Agricultural water, Iran

Prevalence of Anti-*Toxoplasma* IgG seropositivity among Patients Referred to a Clinical Laboratory in Isfahan, Central Iran

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Introduction: positive levels of anti-*Toxoplasma* IgG shows the history of infection with *Toxoplasma gondii*; however it could not differentiate the acute infections from chronic infections. In this retrospective study, we aimed to collect data about anti-*Toxoplasma* IgG seropositivity of the patients having the test referred to Dr. Sharifi Medical Laboratory, Isfahan, Iran.

Materials and Methods: Five hundred eighty-one patients (39 males and 542 females), having anti-*Toxoplasma* IgG test from May 2017 to May 2019 were included in the study. Most of the patients were young pregnant women (93.39%) with the Toxoplasmosis screening test. The patients' test results, which were obtained from serum levels of anti-*Toxoplasma* IgG test using the automated chemiluminescence immunoassay (LIAISON, Italy), were collected, analyzed and reported.

Results: Of the 581 patients, 122 (21%) and 459 (79%) were anti-*Toxoplasma* IgG seropositive and seronegative, respectively. Considering the sex ratio, 16 (41.02%) and 106 (19.55%) were seropositive male and females, respectively.

Conclusion: The prevalence of the infection in this limited study is somewhat lower than most regions of Iran, which may be related to the arid climate of the city. Considering our previous study on the same population (25.3% seropositivity during 2013-2015), *Toxoplasma* infection rate is decreasing slowly in Isfahan, central Iran; however, this study is mostly on the young women and referrals of one medical laboratory and the results may be biased.

Keywords: *Toxoplasma gondii*, Toxoplasmosis, Iran

Prevalence of *Toxocara* spp. eggs in the Soil of Public Parks in Hamedan, Western Iran

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Background and objectives: *Toxocariasis* is a neglected disease with international public health importance transmitted from animals to humans (zoonosis). It is caused by *Toxocara canis* and *Toxocara cati* which are common gastrointestinal helminths of cats and dogs with prevalences ranging from 1.8 to 78% worldwide. As the human patients especially children commonly occur infection via contaminated soils of public areas, this survey was aimed to provide data on the *Toxocara species* eggs contamination in the soil of public parks of Hamedan city, capital of Hamedan Province in west of Iran.

Methodology: Between April and May 2019, a total of 200 soil samples were collected from 20 public parks from all geographical areas of the city. From each park four to ten soil samples were taken according to the area of the parks i.e. separate samples were collected from every 50 steps. Soil sample of approximately 200 g were collected at a depth of 3 cm, stored in plastic bags and taken to the laboratory. For detection of eggs, soil samples were dried overnight at room temperature and sifted through a 150 µm mesh sieve. About 2 gm of powdery soil was subjected to a normal sucrose flotation method. Light microscopic examinations by trained personnel were carried out and photomicrographs were taken from positive samples.

Result: Microscopic evaluation of specimens revealed that 14 samples (11.67%) collected from ten public parks in Hamedan (50.0%) were contaminated with *Toxocara species* eggs. Intensity of contamination ranged from 12.5% to 50.0% in ten parks. All three developmental stages of *Toxocara* spp. eggs (monocell, 2 cells to pre-embryonated and embryonated) were observed. Furthermore, in two public parks *Trichuris*-type eggs were found in the soils.

Discussion and Conclusion: This study provides the first information about contamination of public areas in Hamedan Province and suggests that a public awareness about this fact is needed. Education of citizens especially parents with children, who have commonly mouthing behavior, is recommended. Further studies in the province especially with aid of molecular-based studies for identification of parasites species is suggested.

Keywords: Toxocarisis, Zoonosis, Soil-transmitted helminths, Public area, Education

Ixodid Ticks infesting Dogs in Torbat-e-Heidareih and Mashhad areas, Khorasan Razavi province. Iran

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Background and objectives: A survey was carried out to investigate the prevalence of hard tick species on dogs in Torbat-e-Heidareih and Mashhad areas from 2018-to 2019.

Methodology: A total of 677 ixodid ticks were collected from 86 infested dogs during activating seasons of ticks. Adult ticks were identified under a stereo-microscope according to general identification keys.

Results: Two ixodid species as *Rhipicephalus sanguineus* (57 %) and *Rhipicephalus turanicus* 42.7% were identified in infested dogs. *R.sanguineus* (87%) was dominant tick in stray dogs, while *R. turanicus* (80%) was dominant tick in sheepdogs.

Discussion and Conclusion: The results showed that *R.sanguineus* and *R. turanicus* were important ixodid ticks in dogs in the areas and should be done more studies about role of ticks in transmission of blood parasites.

Keywords: prevalence, Ixodid tick, dogs, Iran

The Study of BDNF Gene Expression in Brain Tissue of Rat Infected to Acute and Chronic Toxoplasmosis: A Study in Animal Model

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Background and objectives: *Toxoplasma gondii* is one of the most common parasitic infections of humans and animals with a worldwide distribution. This protozoa is able to infect all warm-blooded animals. Brain-Derived Neurotrophic Factor is a protein encoded by a gene called BDNF. This protein promotes the development of the peripheral and central nervous system. Many evidence suggests that BDNF plays important roles in memory, learning, behavioral disorder, energy intake and, energy metabolism. In some studies, infection with *Toxoplasma* has been shown to alter the level of BDNF in the brain. The aim of this study was to determine the effect of acute and chronic infections of Toxoplasmosis on the expression of BDNF gene in rat brain tissue.

Materials and Methods: Sixty adult male Wistar albino rats (230-300 g) were allocated into the six groups. Three groups inoculated by 200000 tachyzoite of *T. gondii* RH strain and three groups were controls without inoculation. After two, five and ten weeks, groups 1, 2 and 3 and their controls' brain removed.

Total RNA from the brain was extracted using RNAX_Plus Cinnagen kit according to the manufacturer's instructions and then cDNA synthesized (Fermentas Co). In the next step, primers designed for the BDNF gene, as well as the beta-actin gene, were used as a housekeeping gene for control and comparison in the real time PCR reaction with cyber green fluorescence color.

Results: The expression of BDNF gene amount in the acute infection group was 2.91 however, in the chronic infection groups (5 and 10 weeks infections) was 0.091 and 0.395 respectively that reduction was significant statistically ($P < 0.01$). Acute *T. gondii* infection could reduce the expression of the BDNF gene up to 7.54 fold compared to the control group. There was no significant difference in the expression of the gene in the five-week and ten-week chronic groups compared to the control groups. By inoculating the brain suspension into mice peritoneum and microscopic examination of brain tissue, the parasite was not able to produce tissue cysts.

Conclusion: The results showed that acute infection with RH strain of *T. gondii* can reduce the amount of BDNF expression in the rat brain, while chronic Toxoplasmosis infection has no significant effect on BDNF gene expression.

Keywords: BDNF, gene expression, Acute Toxoplasmosis, Chronic Toxoplasmosis

Frequency and Identification of *Acanthamoeba* spp. in Water Sources of Urmia, West Azarbayjan Iran

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Introduction: Free living amoeba, due to the fact that they can cause important diseases such as keratitis and meningoencephalitis, being studied more precisely in the world. In Iran, many studies have been carried out in most parts of the country or are under investigation. Because previously no study performed about this parasite in West Azerbaijan (north west Iran), the aim of this study was determination of frequency and genotype of *Acanthamoeba* spp. in water sources.

Martials and Methods: A total of 60 water samples were collected from surface and plumbing waters from five regions of Urmia. Samples after filtration cultured in non-nutrient medium in 30°C. and amoeba harvested and DNA extracted. PCR in 18SrDNA fragment performed using primers JDP2 and JDP1, and 11 products sent to sequencing. Results analyzed with bioinformatics software's and submitted in Genbank.

Results: Of the 60 superficial and plumbed water samples, 21 samples were positive, of which four were plumbed water and 17 of them were surface water. Out of the twenty-one positive cases, 10 cases were confirmed in validated centers in terms of gene sequencing. Of the ten cases, one was a T2 genotype and nine were T4.

Discussion: Studies in other parts of the country show that the dominant genotype in Iran is T4, and the frequency and of genotypes of *Acanthamoeba* spp. in Urmia also partially relates to the parts of country.

Keywords: Frequency; *Acanthamoeba* spp; Iran

Down regulation of IL-10R1 and IL-10R2: a Possible Strategy by which the Intermediate Host Struggles to Control *Toxoplasma gondii* Infection

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In this study 50BALB/c inbred mice were divided into 5 groups, four of which, considered as test groups, received separately excretory/secretory product (ESP) from cell culture medium, the ESP from cell free medium, *T. gondii* lysate product (TLP), and live tachyzoite. The fifth group was considered as control and received phosphate buffered saline (PBS). Then, the mice peritoneal leukocytes were collected separately and their total RNA was extracted, converted to cDNA and the gene expression level of IL-10R1 and IL-10R2 was evaluated for the test groups using Q-RT-PCR method relative to the control group. The findings showed a significant statistical difference for the gene expression level of IL-10R1 in the groups of the ESP from cell free medium ($P=0.032$) and live tachyzoite ($P=0.048$). Relating to IL-10R2, the difference of the gene expression level was significant statistically for the groups of the ESP from cell culture medium ($P=0.044$) and the live tachyzoite ($P=0.012$). The gene expression level of IL-10R1 and IL-10R2 was also not significant for the other groups ($P>0.05$). Taking into consideration that *T. gondii* increases the production of IL-10, therefore, high production of IL-10 is incompatible with the down regulation of its receptors (IL-10R1 and IL-10R2), and consequently, the supposition here is that probably the host immune system struggles to decrease the gene expression level of IL-10R1 and IL-10R2 in the infected cell to limit the parasite biological activities.

Keywords: IL-10R1; IL-10R2; *Toxoplasma gondii*; Gene expression

Prevalence of Cutaneous Leishmaniasis in Jiroft City in Southern Iran, 2018-2019

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Introduction: Leishmaniasis is a parasitic infection endemic in about ninety countries of the world. Cutaneous Leishmaniasis is a most common form of the infection caused by several species of *Leishmania* protozoan parasites. Annually, about 1 to 1.5 million new cases of cutaneous Leishmaniasis are reported, worldwide. This disease in Iran are seen in two forms including: dry (urban) and wet (rural). In Iran, about 20 thousand cases of cutaneous Leishmaniasis are reported each year. The distribution and prevalence of Leishmaniasis differs from region to region. The city of Jiroft has a warm, humid climate that provides favorable conditions for the growth of the parasites. So this study was done to investigate the prevalence of cutaneous leishmaniasis in Jiroft city in southern Iran.

Materials and Methods: This cross-sectional study was conducted in 2018- 2019. Skin biopsies were obtained from cutaneous leishmaniasis patients referred to the central laboratory in Jiroft city in southern Iran. All samples were examined by skilled technicians by direct microscopic examination.

Results: A total of 78 patients were participated in this cross-sectional study. In 2018, sixty-three people referred to the central laboratory and *Leishmania* parasite was seen 19 (23.8%) of them. In 2019, fifteen people referred to the central laboratory and *Leishmania* parasite was seen 5 (33.3%) of them.

Conclusion: The results of this study showed that Leishmaniasis is common in Jiroft city and health measures need to be taken to reduce the disease.

Keywords: Jiroft·cutaneous Leishmaniasis·parasite

Study on Prevalence of Intestinal Parasites in Jiroft city in Southern Iran, 2019

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Introduction: Intestinal parasites are widely prevalent in developing countries, probably due lack of sanitation, lack of access to safe water and improper hygiene. All age groups can become infected; however, children are the worst affected. It is estimated that approximately 3.5 billion people are infected and that 450 million are ill as a result of these infections. The distribution and prevalence of various species of intestinal parasites differs from region to region due to social and geographical factors. The city of Jiroft has a warm, humid climate and a diverse vegetation that provides favorable conditions for the growth of parasites. So this study was done to investigate the prevalence of intestinal parasites in Jiroft city in southern Iran.

Materials and Methods: In spring 2019, fresh stool samples were collected from patients referred to the central laboratory in Jiroft city in southern Iran. All samples were examined by skilled technicians by direct microscopic examination.

Results: A total of 908 patients were participated in this cross-sectional study. Intestinal parasites were seen in 45 (4.95%). Among the patients, men and women accounted for 62.2% and 37.8%, respectively. *Giardia lamblia* was the most common parasite detected (66.6%) followed by *Entamoeba coli* (33.4%). Of the 17 women, there were 11 (64.7%) with giardiasis and 6 (35.3%) with amoebiasis. Of the 28 men, there were 19 (67.8%) with Giardiasis and 9 (32.2%) with Amoebiasis.

Conclusion: The results of this study showed that *Giardia lamblia* and *Entamoeba coli* are the most common intestinal parasites in Jiroft city and health measures need to be taken to reduce these parasites.

Keywords: Jiroft·intestinal·parasite

Frequency Distribution of *Giardia duodenalis* Genotypes Isolated from Sheep in Isfahan Slaughterhouses Using *High Resolution Melting* (HRM) Technique

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Giardia duodenalis (*Giardia intestinalis*-*Giardia lamblia*) is one of the most common intestinal protozoan of many vertebrates such as humans and wild and domestic animals. Giardiasis is known as a zoonosis disease and consists of eight major A to H assemblages. In this study, due to the zoonosis of the disease and the importance of recognizing the reservoirs of disease in the transmission of disease, control and prevention, the molecular analysis of *Giardia* parasite isolates in sheep in Isfahan region using HRM technique is explored. 18 sheep were reported from 100 sheep, 18% of *Giardia duodenalis*. Microscopic confirmation for the presence of *Giardia* spp. was performed, cysts *Giardia* spp. positive specimens were concentrated using four layered discontinuous sucrose flotation technique (0.5, 0.75, 1, and 1.5 M) and single-layered sucrose solution (0.85 M). PCR amplification were successfully performed on 16 of 18(88.8%) samples, and HRM analysis was performed by software and sequencing, which showed the presence of genotype assemblage E of *G. duodenalis*.

Keywords: *Giardia duodenalis*, intestinal protozoan, sheep, HRM. Assemblage E

The First Pathological Report of Abomasal Coccidiosis in a Sheep from Razavi Khorasan Province

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Background and aim: Abomasal coccidiosis has been reported in sheep and goats from different countries of the world. Generally, these infections are found incidentally and characterized by the presence of giant Schizonts in the abomasum mucosa. In a few studies, duodenum is also affected by *Eimeria (Globidium) gilruthi*. To our knowledge, there is no report on abomasal coccidiosis from Iran and this study describes gross and histopathological findings of the infection.

Materials and methods: Abomasal samples of a female, 3-year-old sheep was referred to Pathology Department due to proliferative lesions. The clinical diagnosis was ostertagiosis. For microscopical examination, the samples were fixed in 10 percent buffered formalin and processed according to the standard histological techniques for paraffin embedding. Sections of 5 micrometers thickness were cut and stained with conventional hematoxylin and eosin (HE) method.

Results: Macroscopically, proliferative lesions were found on the mucosa of the Abomasum. Histopathological examinations of the lesions revealed a couple of megloschizonts within the tunica mucosa of the affected abomasum. These schizonts contained a lot of merozoites and had thick cyst walls. A mononuclear inflammatory reaction was observed around the schizonts.

Conclusion: According to gross and histopathological characteristics, the disease was diagnosed as abomasal coccidiosis and in case of abomasal ostertagiosis, coccidiosis should be considered as differential diagnosis.

Keywords: Abomasal coccidiosis, ostertagiosis, sheep.

Frequency of intestinal parasites in diarrheic patients admitted to ShahidRajaeepolyclinic in Ahvaz County, southwestern Iran

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Background: Intestinal parasitic infections are a public health problem and the main cause of morbidity and mortality in endemic regions. Despite the efforts to improve health status, intestinal parasitic diseases still remain a global public health problem, especially in developing countries such as Iran. The main objective of the present study was to investigate the intestinal parasitic infection in diarrheic patients referred to ShahidRajaeepolyclinic in Ahvaz County.

Methods: This cross-sectional study was conducted from 2017 to 2019 among 250 diarrheic patients admitted to the ShahidRajaeepolyclinic in Ahvaz County. The collected stool samples were investigated using direct smear, formalin-ether concentration, modified Ziehl-Neelsen, and Trichrome staining techniques.

Results: Out of the participants, 43.6% were female and 56.4% were male. The age range was 1-70 years. The overall prevalence of intestinal parasites was 39.2%, which 36.4% was infected with at least one pathogenic intestinal parasite. *Giardia duodenalis* (18.8%), and *Blastocystis hominis* (15.2%) were the most frequently observed protozoan.

Based on trichrome staining and modified Ziehl-Neelsen *Entamoeba histolytica/dispar* and *Cryptosporidium* spp. were reported in 3/6% and 2/8% patients, respectively. Co-infections with two intestinal parasites were observed in 4% cases and in one case, infection with three intestinal parasites was found. In this study, helminthic infection was not observed.

Conclusions: The results of the present study revealed that *Giardia duodenalis* was the most common cause of infection in investigated patients. It seems that bacterial and viral agents are also involved in gastrointestinal diseases.

Keywords: Intestinal parasites, diarrheic patients, Ahvaz, Iran

Study of dissemination of cutaneous Leishmaniasis in Shoushtar city, Southwest of Iran, during the last decade

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Introduction: Cutaneous leishmaniasis (CL) consist two type containing zoonotic CL (ZCL), as a result of *Leishmania major*, and anthroponotic CL (ACL) that it causes with *Leishmania tropica*, as protozoa parasites, is widespread in the world specially in the almost City of Iran. Demonstration of disease is skin lesions comprise ulcers on points of the body that's exposed to biting of sand fly, as vectors, also permanent scars and the serious damage that named stigma. This investigate was extracted among of dates of patients referred to the Health center Shoushtar City at latest ten years.

Material and Methods: This investigate was conducted on all of the 929 doubted CL that referred to Health Centers of Shoushtar city in the southwest of Iran, from 2009 to 2019. Dates of the last ten years have been collected and so analyzed. First bodily checks were carried out on skin of referrals to the Health Center and prepared smears were fixed with absolute methanol and stained with Giemsa then CL cases were confirmed by microscopical examinations. All the Giemsa-stained slides examined under a light microscope with high magnification.

Results: Our findings indicated that from 929 total prepared sample's CL cases, 420 (45.2%) of them were microscopically positive to occur in the last decade in Shoushtar city. The most frequent of CL was observed in 1393 (20%) and 1394 (19.2%), also the low level was seen in 1392 (3%). Results completely showed in the below table. Table 1, Results of investigating on dates patients who referring to the Health center of Shoushtar City during 2009-2019.

Discussion & Conclusion: Based on analysis achieved dates of suspected CL to Shoushtar Health Center in recent 10 years first, the most monitoring of CL lesions were observed on hands (42.8%), feet (31 %), and face (11.8%) and mix contamination (14.3%) respectively, also highest frequency of CL was observed in men 57.8% more than women, 42.1% that it means most infections were in point of body that exposed to biting of Phlebotomus so regard to the areas as an endemic focus (1, 2) and having two agents, rodent host and phlebotomus also surrounded rural regions that they can play a vital role in the incidence of Leishmaniasis then it needs to further study such as molecular epidemiology to determine differential species in hosts and reservoirs with advance method biochemical, immunological and other investigates on failure drug cases as point important *Leishmania* infection that it help to more effective control of *Leishmania* and prevent of spread it in surrounding on the other hand attention to flood in initiate of current year together with increase kind of insect special sand fly is important for increasing prevalence of disease.

It is an important high dissemination relatively of Leishmaniasis in Shoushtar City also, consideration to many suburbs that surrounded Shoushtars' in Geographic map so referees are resident there and population.

Keywords: Cutaneous Leishmaniasis, *Leishmania* species, Shoushtar

Epidemiology Hydatid Cyst in Imam Khomeini Hospital in Shirvan, North Khorasan Province during 2015-2017

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Abstract

Background: Hydatidosis is one of the most important zoonotic diseases with global distribution. the study aimed to determine the surgical cases of hydatid cyst in Imam Khomeini Hospital in Shirvan, North Khorasan Province during 2015-2017.

Methods: In this descriptive study, the case of 25 patients with hydatid cyst in Imam Khomeini Hospital in Shirvan during the period of 2015-2017 was selected. Patients' demographic information was extracted from their medical records.

Results: Of the 25 patients underwent surgery, 13 cases were related to 2015 and 10 cases related to 2016 and 2 cases related to 2017. From a total of all patients, 80% and 20% patients were females and males respectively; 76 % patients were resident in rural areas and 26% in urban areas. prevalence of the hydatid cyst was in the liver (92%) and in the lung (8%). Among the methods of imaging, sonography and CT scan had diagnosis of the disease .

Conclusion: Creating appropriate methods to prevent the disease is considered a health. identification of the populations most at risk and educating the community about the most common modes of acquisition could be helpful in the control and prevention of this disease.

Keywords: Hydatid cyst, Epidemiology, Shirv

Determining the frequency of cystic Echinococcosis among suspected cases referred to health centers southwest Iran, and post-treatment serologic follow up

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ABSTRACT

Introduction & Aims: Cystic Echinococcosis (CE) is a zoonotic disease caused by the larval stages of *Echinococcus granulosus*. This study was designed to determine the prevalence of hydatidosis in Khuzestan Province and to evaluate the antibody changes in infected individuals after treatment.

Methods: A total of 454 sera were collected from health centers of Khuzestan Province (from 2013 to 2018). Demographic data such as age, gender and history of disease were recorded. Serum samples were investigated for antibody against CE by ELISA using antigen B. Thirty six of cases were followed up after treatment.

Results: Among the 454 evaluated cases, antibody against CE was detected in 184 (40.52%) including 115 (62.5%) females and 69 (37.5%) males. Age distribution was from 8-97 years, the highest prevalence of hydatid cyst was observed in age group 40-49 years.

Liver was the most infected organ (76.63%). Relapse of CE occurred in 23 of patients. In the majority of patients the antibody decreased, whereas in some cases increased CE antibody observed during post-treatment follow up.

Conclusion: Current study indicated the high prevalence of hydatidosis and rate of relapse after treatment among suspected patients. Therefore, long periods and regular follow-up of patients after treatment is necessary and for these monitoring, antibody assay can be an appropriate method.

Keywords: cystic Echinococcosis, hydatid cyst, ELISA, Iran, antibody detection

Frequency of intestinal parasites in patients referring to the laboratory of the Persian Gulf Hospital in the Hormozgan 2017-2018

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Introduction: Parasitic infection is among the most important health problems around the world, especially in developing countries. Regarding the importance of the epidemiology of parasitic diseases and the necessity of determining the baseline of parasites in different regions and communities, the present study has been conducted to determine the frequency of intestinal parasites in patients referred to the Gulf Hospital Laboratory of Social Security in Hormozgan province.

Materials and Methods: In a cross-sectional study report of stool exam was evaluated for all referred sample to the laboratory. The reports was based on direct microscopy and collected data reported with descriptive statistics with percent.

Results: 4000 reports were studied in which 2250 (56.2%) were male and 1750 (43.7%) were female. The highest number of positive cases related to the autumn where as the lowestone related to for the winter. The number and frequency of pathogenic and non-pathogenic intestinal parasites were *Giardia lamblia* 55 (1.37%), *Blastocystishominis* 61 (1.52%), *Entamoeba histolitica* 2 (0.05%), *Entamoeba coli* 68 (1.7%), *Chilomastixmesnili* 11 (0.27%), *Endulimax Nana* 10 (0.25%), *Trichomonashominis* 3 (0.075%), *Hymenolipisnana* 1 (0.025%), *Ascarislumbricoides* 2 (0.05%), *Taeniasaginata* 2 (0.05%) were determined.

Conclusion: The prevalence of parasites in this study is lower than in comparison with other studies. The findings of this study indicate that the frequency of intestinal parasites in Hormozgan province is decreasing due to warm climate and increased health condition. However, as in other parts of the country.

Keywords: protozoa, helminthes, intestinal parasite, infection disease.

Pharynx Leishmaniasis: A Rare Case of an Unusual Clinic Pathological Entity in Iran

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Background and aim: Protozoan parasites of the genus *Leishmania* cause a wide spectrum of clinical manifestations known as Leishmaniasis, which affect millions of people worldwide. Visceral leishmaniasis (VL) has been reported sporadically in Iran, but the disease is endemic in northwestern and southern areas.

Materials and Methods: In November 2014, a 37-years-old man with a symptom of pharyngeal lymphadenopathy was referred to Masih Daneshvari Hospital in Tehran, Iran. He had a history of positive bone marrow microscopic examination and was treated with meglumine antimoniate and liposomal amphotericin B, 8 years ago. At the first, a biopsy was prepared and blood sample was taken for serological tests and were transferred to the laboratory of Leishmaniasis, School of Public Health. Then, the biopsy sample was cultured in NNN and RPMI-1640, serological direct agglutination test (DAT), Nested-PCR and sequencing were performed.

Results: One month after the microscopic examination of the culture medium, the promastigotes did not grow and the culture result was negative. The result of DAT was positive (1/51200 titration). Molecular diagnosis (Nested-PCR) of the sample confirmed *Leishmania infantum* and sequencing analysis confirmed Leishmaniasis caused by *Leishmania infantum*.

Conclusion: A rare case of pharynx Leishmaniasis was confirmed by serological and molecular assays in Iran. Diagnosing of oral Leishmaniasis presents a challenge but early diagnosis is necessary to provide prompt treatment and to avoid recurrences.

Keywords: *Leishmania infantum*, Human, Iran.

Differentiation of *Toxocaracanis* and *Toxocaracati* in Fecal Samples from Household and Shelter Dogs and Cats Based on Parasitological and Molecular Analysis

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Introduction: Toxocariasis is an important zoonotic disease caused by the second-stage larvae of *Toxocara canis* and *Toxocara cati*. The purpose of this study was to differentiate the infection caused by these ascarids in cats and dogs, using stool exam and PCR analysis methods.

Material and Methods: Fecal samples collected from 9 household cats and dogs, and 7 animals kept in the animal shelter. Then samples examined for *Toxocara* spp. By sedimentation and flotation with ZnCl₂-solution. In *Toxocara* positive samples, *Toxocara* eggs were microscopically measured utilizing 10×. Genomic DNA from the eggs of *Toxocara* spp. was extracted using MBST kit. In the present study, PCR amplification of the COX1 cytochrome c oxidase subunit 1 was used to identify the eggs of *Toxocara* spp. The results were confirmed by Sanger sequencing.

Results : Of the 132 fecal samples that were randomly collected from household dogs, and animals kept in the animal shelter were not found *Toxocara* spp. eggs. 30 samples of household cats were studied in this study, which reported 3.3 percent of the infections. Eggs of *Toxocara* spp. in feces of stray cats were detected by the ZnCl₂ flotation method, and identification was conducted by polymerase chain reaction (PCR) and DNA sequencing. Identification of *Toxocara* spp. using molecular methods is sufficiently sensitive to detect low levels of parasites and identify the different *Toxocara* spp. in feces. The results of this study indicate the efficiency of antiparasitic drugs used in the treatment of domestic cats and dogs. It also shows that domestic dogs and cats are not considered a risk factor of Toxocariasis for their owners.

Keywords: dogs, cats, *Toxocara*, cytochrome c oxidase subunit 1, ZnCl₂ flotation method

Phylogenetic analysis of *Hymenolepis nana* isolates from human based on ITS2-rDNA gene marker

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Background: *Hymenolepis nana* is a common worldwide-distributed cyclophyllid enteric parasite of human, leading to severe infection in immunodeficient patients.

Due to the presence of a morphologically similar cestode, namely *Rodentolepis nanai* in rodents, the molecular study of this parasite is a subject of importance. In the present study, isolates of *H. nana* with human origin were phylogenetically analysed.

Methods: Genomic DNA of *H. nana* eggs were extracted from stool samples of two infected patients. Both patients were residents of Iran, but one of them was originally from Afghanistan. PCR amplification of ITS2-rDNA region was performed, and products were sequenced. Phylogenetic analysis was carried out using MEGA 7.0 software.

Results: Blast analysis showed that the present two isolates of *H. nana* had 100% homology with each other and with the isolates of *H. nana* of human and *Rhombomys opimus* from Iran, *Rattus norvegicus* and *Mus musculus* from Japan and *Rattus rattus* from India. Phylogenetic analysis indicated that the current isolates were placed in the same branch of *H. nana* isolates from other areas of the world, clearly separating from *Hymenolepis diminuta*.

Conclusion: Based on ITS2-rDNA phylogenetic analysis, *H. nana* isolates are distinctive from *H. diminuta*, but identical to each other irrespective of their human or rodents host of origin.

Keywords: *Hymenolepis nana*, human, ITS2-rDNA, Iran

Investigation of *Zataria multiflora* Nano-emulsion Extract on *Echinococcus granulosus* Protoscoleces and Microcysts *in vitro*

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Background and objectives: Cystic hydatidosis is one the most important zoonosis caused by larval stage of *Echinococcus granulosus*. Herbal remedies such as *Zataria multiflora* extract are recommended as protoscolicidal agents. In this study the effect of *Z. multiflora* nano-emulsion was evaluated on *E. granulosus* protoscoleces and microcysts *in vitro*.

Methodology: hydatid cyst fluid contains protoscoleces was aspirated from infected sheep liver. Protoscoleces were exposed to nano-emulsion and emulsion of *Zataria multiflora* at the concentrations of 1, 2, 5, 10, 15 and 20 mg/ml. Albendazole (5mg/ml), normal saline and nano-emulsion without essential oil of *Z. multiflora* served as control groups. The mortality rate of protoscoleces was determined by an optical microscope using eosin- exclusion test. Developed microcysts of long-term cultured protoscoleces were treated with *Z. multiflora* nano-emulsion optimal concentrations and investigated by inverted microscope.

Results: *Z. multiflora* emulsion at a concentration of 20 mg/ml for the duration of 15 minutes, and nano-emulsion for the duration of 10 minutes, resulted in a 100% death of protoscoleces. Additionally, densely packed aggregates were formed inside the microcysts treated with *Z. multiflora* nano-emulsion and emulsion at a concentration of 20 mg/ml after 40 minutes, but complete destruction of laminated layer did not occur.

Discussion and conclusion: The results of this study indicate that *Z. multiflora* nano-emulsions had a higher anti-protoscoleces effect than its emulsion, but these two compounds had a similar degenerating effect on microcysts.

Keywords: *Echinococcus granulosus*, glucose, development, microcyst, *in vitro*

Characterization of *Toxoplasma gondii* Genotypes Isolated from Slaughtered Goats

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Introduction and objectives: *Toxoplasma gondii* is transmitted through various sources, such as drinking water as well as eating undercooked or raw meat and congenital infections. Since goat meat plays an important role in the human diet in Iran, this study aimed to determine the prevalence of *Toxoplasma gondii* among goats and identification of isolates by genotyping.

Methods: Briefly, a total of 561 samples consisting of heart, diaphragm, and tongue tissues were collected from 178 slaughtered goats in the Jahrom abattoir located in Fars province from January to April 2017. A Nested-PCR assay was performed to detect *Toxoplasma gondii* using the GRA6 gene as a target. Genotyping was done by Nested PCR-RFLP based on GRA6 and SAG2 Genes.

Results: The prevalence of *Toxoplasma gondii* infection among goats was found to be 18.2%. The highest rate of infection was found in diaphragm tissue (35.4%) followed by heart (26.5%) and tongue (14.7%). Co infections of heart with diaphragm, tongue with the diaphragm and heart with tongue were found in 17.6%, 2.9% and 2.9% of goats, respectively. In terms of genotype, the highest frequency was related to type I *Toxoplasma gondii* (58.8%).

Discussion and conclusion: In this study, the highest prevalence among infected goats is related to genotype I. Since this type of parasite is highly pathogenic and goat meat is a major source of human nutrition in Jahrom, it should be considered to implement preventive control programs in order to avoid further infections.

Keywords: *Toxoplasma gondii*, goat, Genotype, Jahrom

Viability and Infectivity of Tachyzoites of *Toxoplasma gondii* exposed to Butanedione monoxime

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A wide range of vaccine strategies have been investigated for *Toxoplasma* infection. Penetration of *Toxoplasma* tachyzoites into the host cells can be inhibited by Butanedione monoxime (BDM) as an inhibitor of myosin-actin interaction. The aim of this study was to investigate the effects of this molecule on the viability and infectivity of *Toxoplasma* tachyzoites to provide a new option for the vaccine development.

In this study mortality effect of different concentrations (1, 2, 4, 8, 16, 32, 64, 128 µg/mL) of BDM on RH strain of the tachyzoites was assessed by flow cytometry. The ability of penetration of the tachyzoites exposed to the different concentrations of BDM was investigated in HeLa and J774 cell lines. To evaluate the infectivity, 2×10^5 tachyzoites exposed to the different concentrations of the compound were subcutaneously injected into 50 mice.

The highest mortality was seen in tachyzoites exposed to 128 µg/mL, that was 72.69%. Tachyzoites which were exposed to 32, 64, and 128 µg/mL of BDM, begun the proliferation in HeLa cell after 48 hours while this proliferation initiated within 24 hours in macrophage cell. All of the mice, inoculated with the BDM-treated tachyzoites were died after 13 days. The survival means of the mice receiving tachyzoites exposed to 128 µg/mL of BDM was 12.4 days which significantly differed from the negative control group.

In conclude Besides the inhibitory effects of Butanedione monoxime represented in cell invasion of *Toxoplasma gondii*, this effect immediately decreased. It seems that BDM is not a suitable candidate for production of vaccine against Toxoplasmosis.

Keywords: Butanedione monoxime, *Toxoplasma gondii*, Cell Culture

Morphological and Molecular Study (ITS1) of Hydatid Cysts in the Slaughtered Sheep and Goats in Rasht Region

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Introduction: Hydatidosis is a common disease of humans and livestock caused by *Echinococcus granulosus* which is more common in Iran. It has 10 strains, G1 to G10, which are different in morphology, epidemiology, treatment, control and also in their interfaces. The main objective of the present study is to investigate the morphological and molecular characteristics of Hydatid cysts in sheep and goats at the slaughterhouse of Rasht.

Material and methods: In this study, 50 infected livers were transferred to the parasitology laboratory. After extraction of fluid cysts, morphological indices of rosetal protoscolex hooks were measured by using an ophthalmic lens.

Results: Based on the results of T-test for the samples of sheep and goats, it can be concluded that there is a significant difference between them in terms of some morphological characteristics. In the molecular part of this study, after DNA extraction, the PCR reaction was performed on the ITS1 gene. The PCR product was then digested by the Bsh1236I enzyme. The samples isolated from sheep and goats showed the strain G1 - G3.

Discussion and conclusion: Iran is one of the most important breeders of sheep and goats in the world and can play an important role in the *Echinococcus* parasitic cycle. According to the current study, the highest strain found in sheep and goat is G1 strain which should be considered in control program.

Keywords: *Echinococcus granulosus*, hydatid cyst, PCR, goat, sheep, Rasht

Association of *Toxoplasma gondii* infection with cardiovascular diseases: a cross-sectional study among patients with heart failure diseases in Urmia, North-West of Iran

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Objectives: For the first time, the current study aimed at assessing the relationship between cardiovascular disease and Toxoplasmosis among cardiac patients attended a cardiology center in Urmia, Iran.

Methods: This study investigated the seropositivity rate of anti-*Toxoplasma* IgG antibodies by ELISA in 375 patients with cardiovascular diseases and 336 healthy volunteers.

Results: The seropositivity rate of anti-*Toxoplasma* IgG antibodies was significantly higher in cardiovascular patients (63.73 %) than in healthy volunteers (37.64%) ($P < 0.001$). Also, a positive association was observed between anti-*T. gondii* IgG antibody seropositivity and cat contact ($P \leq 0.001$, OR: 5.178; 95% CI: 1.97-13.57), consumption of raw or undercooked meat ($P \leq 0.001$, OR: 0.3; 95% CI: 0.15-0.61), and consumption of unboiled milk ($P \leq 0.001$, OR: 0.26, 95% CI: 0.12-0.54).

Conclusion: Our findings indicate a potential association between *T. gondii* infection and heart disease and suggest that heart disease might be related with a chronic infection. Thus, parasitological screening of patients with heart diseases is required periodically to prevent the possible dissemination of toxoplasmosis. Risk factors associated with *T. gondii* exposure are critical to design future prevention strategies against *T. gondii* exposure.

Keywords: Cardiovascular Diseases, Frequency, Heart failure, Toxoplasmosis

The survey of parasitic diseases of the honey bees (*Apis mellifera*) and its association with Colony Collapse Disorder (CCD) in apiaries of Talesh

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Introduction: Honey bee (*Apis mellifera*) because of its production including honey, royal jelly, wax, venom, propolis, and also its role in pollination is one of the most economically beneficial insects worldwide. In recent years, honey bee colonies have been suffering high mortality due to several factors such as viral, parasitic diseases and etc.

Aims: The aim of this study was investigation of parasitic diseases and its association with CCD in honey bees.

Methods: Totally 40 apiaries were selected from various regions of Talesh in every season. They were divided into two groups, involved with CCD and healthy, and then 10% of the hives of each apiary were selected for examination. Detection of different parasites agent were examined by routine methods. The specific primers of 16S rRNA were used for distinguishing of *Nosema apis* and *N. ceranae*.

Results: The rates of CCD in examined apiaries were 87.5%, 0.025%, 10% and 95% in the seasons of spring, summer, autumn, and winter, respectively. Also, *N. ceranae* infections in the mentioned seasons were 87.5%, 47.5%, 65% and 85%, as well as infections with *Varroa destructor* were 10%, 55%, 47.5%, 12.5%, respectively. However, in any season of the year, there was no infection with *Acarapis woodi* in the examined apiaries. The statistical analysis showed that a significant correlation between *Nosema* and CCD in winter and spring ($P < 0.001$) but the statistical analysis did not show a significant relationship between *Varroa* infection and CCD.

Discussion: *Nosema* infection might be one of the causes of CCD in the bee populations under study.

Keywords: Honey bee, *Nosema apis*, *Nosema ceranae*, *Varroa destructor*, Colony Collapse Disorder, Talesh

Using of activatable melittin as an prodrug for targeting protease activity in cutaneous Leishmaniosis

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INTRODUCTION: Cutaneous Leishmaniasis (CL) is now known as a major problem in many developed and undeveloped countries around the world. Most of the commonly used anti Leishmanial drugs, such as pentavalent antimonial agents, exhibit considerable toxicity, and also there are reports of large-scale clinical drug resistance in Leishmaniasis. Cell penetrating peptides like synthetic activatable melittin, could be designed as a suitable alternate drug.

Aim: the aim was designing of an inhibitor poly anionic peptide consisting of nine glutamic acid (E9) attached to melittin via a Matrix Metalloproteinase (MMP) degradable linker for selective targeting of *Leishmania* infected cells. So when synthetic activatable melittin is entered to the infected cell, due to enough MMP-2 enzyme, cleavage of the linker and the ensuing release of inhibitor peptide E9 occur and melittin would be reformed as an active shape.

Methods: Melittin is 26 amino acid peptide with no disulfide bridge; the N-terminal part of the molecule is predominantly hydrophobic and the C-terminal part is hydrophilic and strongly basic. The sequence of melittin was retrieved from NCBI and E9 and MMP cleavable linker was attached to it (EEEEEEEEEGPVGLIGKGIGAVLKVLTTGLPALISWIKRQQ). Folding of this construct predicted by pep folds 3 servers.

Results: We assumed that melittin with highly cationic carboxyl terminus may be inhibited when connected to E9 via a specific cleavable linker for MMP-2 like GPVGLIGK.

Discussion: some modifications of melittin for selective killing of involved cells is necessary for therapeutic application.

Keywords: *Leishmania*, Melittin, Matrix Metalloproteinase

Mass spectrometry analysis of ~27/28 kDa Hydatid Cyst Wall Antigens and its anti-breast cancer effects

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Abstract

Introduction and objective: Cytotoxic agents that used to kill cancer cells usually result in undesirable problems. Immunotherapy has fewer side effects; however, poor immunogenicity of cancer antigens often fails to induce a satisfying immune response. Employing parasite antigens including hydatid cyst wall (HCW) antigens for anti-tumor immunization can induce a significant anti-tumor immune response due to antigen homology and cross-reaction. Therefore, our aim was to evaluate anti-tumor effects of 27/28 KDa HCW antigens in mice immunized with these antigens.

Methods: HCW antigens were probed with breast cancer patients' sera. Mice were immunized with the reactive antigens and then injected by 4T1 cancer cells. The mice tumors' growth, metastasis, and mice survival were assessed. MALDI TOF MS was employed to assess homologies between antigens of parasite and cancer cells.

Results: Some breast cancer patients' sera cross-reacted with the ~27/28 kDa protein bands of HCW antigens. BALB/c mice immunization with the antigens could inhibit the growth and metastasis of 4T1 breast tumors, which resulted in a significant increase in tumor-bearing mice survival. Mass spectrometry analysis showed that 27/28 kDa protein bands composed of several proteins that some of them had a high homology with cancer cell antigens.

Discussion and Conclusion: our results indicated that the homology between antigens of produced by breast tumors and HCW can be a positive factor for the usage of 27/28 kDa protein bands of HCW antigens to activate immune system of patients against breast tumor.

Keywords: Hydatid cyst, 4T1 Breast cancer, Mass spectrometry, Tumor, Metastasis, Immunization

Cutaneous Leishmaniasis and microbiota

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Introduction: Cutaneous Leishmaniasis (CL) is caused by intracellular protozoan parasites and is characterized by a spectrum of clinical manifestations, ranging from self-healing single lesions to chronic, and in some cases metastatic, lesions. Recently, different studies are focused on the association between cutaneous leishmaniasis and changes in the microbiota of skin. A microbiota is an ecological community of commensal, symbiotic and pathogenic microorganisms found in and on all multicellular organisms. In this review association between CL and skin microbiota is discussed.

Materials and methods: The selection of studies in this review was based on searches in PubMed, Library of Congress, Web of Science, Scielo, and Google Scholar, with no specific year range. The search strategy included combinations of the following Keywords: Leishmaniasis and microbiome, skin microbiota and *Leishmania*, cutaneous leishmaniasis and skin microbiome, *Leishmania* and microbiome and *Leishmania*, microbiome and metagenomic analysis.

Results: Salgado et al., (2016) results showed that cutaneous microbiological signatures in leishmaniasis lesions from patients infected with *L. braziliensis* exhibit restricted bacterial diversity compared to those of healthy skin. They also observed that anaerobes, microaerophiles, and facultative anaerobic bacteria were most frequently detected in CL, with a reduction of the aerobic bacteria in normal skin. Gimblet-Ochieng (2017) found that the *L. major* infection in mice, as well as, *L. braziliensis* infection in humans caused a dysbiosis in the skin microbiota on lesional skin and nearby skin sites, characterized by a dominance of *Staphylococcus* spp. or *Streptococcus* spp.

Discussion: Cutaneous ulcers of leishmanial infection are chronic wounds with an anaerobe-predominant microbiome, further impacted by the immunomodulatory effects of *Leishmania* and superinfecting bacteria.

Keywords: *Leishmania*, Leishmaniasis, Skin, Microbiota

Scolicidal effect of the aromatic water of *Citrullus colocynthis*

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Introduction: Hydatidosis is one of the most important parasitic zoonoses with worldwide distribution. New and natural scolical agents not only may be used for production of new antihydatid drugs, but also, they may be useable instead of existing scolical chemical agents. In the present study, the scolical effect of the aromatic water (AW) of *Citrullus colocynthis* was investigated.

Materials and methods: For AW extraction, one hundred kilograms of plant were hydrodistilled with 400 l water for 3 h, using an industrial apparatus. Protoscolices of hydatid cysts were collected aseptically from livers of naturally infected sheep. Hydatid fluid was aspirated from the cysts and centrifuged. The sedimented protoscolices were washed several times with normal saline under aseptic conditions. Protoscolices were exposed to different concentrations of *C. colocynthis* AW for ten min. Viability of protoscolices was confirmed by 0.1 % eosin stain.

Results: Scolical power of different concentrations (10, 20, 40, 60, 80 and 100%) of *C. colocynthis* AW was 83, 98.7, 100, 100, 100 and 100 % respectively. The death rate of protoscolices was 3 % in the control group. The scolical effect of *C. colocynthis* AW was extremely significant compared to the control groups at all exposure times.

Discussion: This study confirmed that the AW of *C. colocynthis* has clear destructive effect on the protoscolices of hydatid cyst. The possible cytotoxicity of *C. colocynthis* AW and its main components should be more investigated in future in vivo studies.

Keywords: hydatid cyst, *Citrullus colocynthis*, Aromatic water

A Nasal Myiasis in a 24-Year-Old soldier in northern Iran

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Introduction: Myiasis is infection of tissues or organs of animals or Human by fly larvae. This infection frequently occurs in livestock and pets in rural areas. In humans, myiasis occurs primarily in unhealthy individuals in third world countries. Nasal myiasis is a rare condition, with only a few reported cases and no treatment usually.

CaseReport: In April 2019, a 24-year-old soldier who Had a car accidentseverehead trauma was referred the to Razi Hospital in Qaemshahr town, Mazandaran province. The patient was severely damaged by the head. He was admitted to the ICUward. His nose was full of larvae. The patient died before the larvae were taken to the test for diagnosis. The patient probably fell on the corner of the road after a caraccident, and the flying egg penetrated into his nose and later turned into a larva. These larvae probably were *Mosca domestica*.

Discussion: To treatment this type of myiasis, larvae should be removed first. Then, wash with saline solution, Ivermectin solution. ultimately eating of oral Ivermectin. But this patient did not have an opportunity to cure.

KeyWords: Nasal myiasis, *Mosca domestica*, Qaemshahr, Head trauma

Cystic Echinococcosis/hydatid cyst co-infection with HIV: Report from Shiraz, Iran

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Abstract

HIV co-infected with other parasitic diseases may cause a serious problem for the patients. A few case reports describing Echinococcosis with human immunodeficiency virus (HIV) infection have been reported in the world; however, it has not been reported in Iran, so far. Here, the first case of liver hydatid cyst co-infected with HIV in Iran is reported. The patient is a 46-year-old female HIV positive based on laboratory report. Her clinical symptoms included abdominal pain, abdominal enlargement and anorexia. Ultrasound showed three large hepatic hydatid cysts with hundreds of daughter cysts. Ultrasonography of the cyst revealed it as CE2 stage according to the WHO classification. The patient went under complete anesthesia followed by complete cyst removal by surgery. Observation of the hydatid cyst fluid using eosin 0.1 % revealed more than 70% viable protoscoleces. Histopathology examination, polymerase chain reaction (PCR), viable protoscoleces confirmed the diagnosis of Echinococcosis. The IgG ELISA test with native AgB for *E. granulosus* infection was also positive. mtDNA amplification using PCR and sequencing showed the cyst as *E. granulosus* sensu stricto genotype. In conclusion, our observation showed three huge hydatid cysts confirmed by different experiments and co-infected with HIV which can aggravate hydatid cyst or may accelerate cyst growth, producing huge or large cyst with even hundreds of daughter cysts which makes it difficult for complete removal of the cyst, but in any case, surgery is superior.

Keywords: *Echinococcus granulosus*; hydatid cyst; HIV

Molecular and serological detection of *Toxoplasma gondii* in meat of chicken and ducks

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Introduction: Consumption of raw or undercooked meat from infected birds is considered as a major source of *Toxoplasma gondii* infection for humans. Meat juice serology has proven to be an excellent method for detection of *T. gondii* infection at slaughter in different species. The aim of the present study was to determine the serologic diagnosis of *T. gondii* in meat juice of some birds by ELISA method. Furthermore, in order to confirm the presence of the parasite, PCR with specific primers for B1 gene was used.

Materials and methods: For this purpose, meat samples were collected from 50 chickens and 17 ducks. From the collected meats, meat juice for serological study and DNA for molecular study were extracted.

Results: In the serological survey of *Toxoplasma gondii* infection of meat juices, 7 out of 50 chickens (14%) and 3 out of 17 ducks (17.6%) were infected with the parasite. Using PCR, it was determined that 4 out of 50 chicken meat samples (8 %) and 3 out of the 17 duck samples (17.6 %) were infected with *Toxoplasma gondii*.

Discussion: Results of this study showed that there is a risk of transmission of parasites through the consumption of raw or undercooked birds' meats. Therefore, complete cooking of birds' meats should be considered.

Keywords: *Toxoplasma gondii*, ELISA, PCR, Bird

A Review study on distribution of Ticks in Iran

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Background and objectives: Ticks are haematophagous arthropods belonging to the class Arachnids. Once they attach to a host for a blood meal, they could cause skin irritation and anemia. Ticks are also one of the major vectors of pathogens, such as *Babesia*, *Theileria*, and *Anaplasma* spp., to animals in the world.

Methodology: All articles about ticks in Iran searched on various sites (pubmed, googlescholar, sciencedirect, scopus, magiran) for 10 years.

Results: Our result showed that there are 30 articles about distributions of ticks in different geographical area in Iran for 10 years ago. The most Hard and soft ticks in Iran including: *Boophilus annulatus*, *Dermacentor marginatus*, *Dermacentor niveus*, *Haemaphysalis erinacei*, *Haemaphysalis choldokovsky*, *Haemaphysalis inermis*, *Haemaphysalis sulcata*, *Haemaphysalis numidiana*, *Haemaphysalis punctata*, *Hyalomma concinna*, *Hyalomma anatolicum*, *Hyalomma asiaticum*, *Hyalomma asiaticum asiaticum*, *Hyalomma marginatum*, *Hyalomma marginatum marginatum*, *Hyalomma aegyptium*, *Hyalomma detritum*, *Hyalomma anatolicum anatolicum*, *Hyalomma anatolicum excavatum*, *Hyalomma scupense*, *Hyalomma schulzei*, *Hyalomma detritum*, *Hyalomma dromedarii*, *Hyalomma lusitanicum*, *Rhipicephalus bursa*, *Rhipicephalus turanicus*, *Rhipicephalus sanguineus*, *Ixodes ricinus*, *Ornithodoros lahorensis* and *Argas persicus*, respectively.

Discussion and Conclusion: This review study shows that prevalence and distribution of ticks are different and comparable in different regions of Iran. Also, because of changing in climate and ecosystem, it is necessary for surviving more on distribution ticks in other regions of Iran.

Keywords: Ticks fauna, Iran

Hydatid Cyst Epidemiology and Risk factors in Hamedan, Iran: 6-year Evaluation

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Abstract:

Background and Objectives : One of the most important zoonosis, cystic Echinococcosis is endemic in some parts of the Middle East, such as Iran. There are some studies on the prevalence of this infection in animal and human in Iran. This cross-sectional study aimed to evaluate the prevalence of CE in human of Hamedan since 2013 to 2019.

Method: In this cross-sectional study, the Hamedan health center records of all hydatidosis-affected patients admitted in three teaching hospitals of Hamedan Province between 2013-2019 were reviewed. Hydatidosis-relevant demographic characteristics, clinical findings, age, sex, contact with dog, consume contaminated vegetables data were collected. Also, the numbers of infected slaughtered animals in these years, were recorded. The descriptive statistical analysis was performed by SPSS software.

Results: Totally, 98 patients with the mean age of 10-60 yr were admitted with cystic Echinococcosis (CE) diagnosis. Moreover, the highest and the lowest prevalence of CE cases were in age ranges of more than 50 (31.63%) and less than 10 (5.1%) yr, respectively. Male and female infected were 51.02 % and 48.97% . Majority of the cases were urban residents (53.06 %) and 41.83% patients had close contact with dog and consume contaminated vegetables, Simultaneously, but 58.16% only consumed contaminated vegetables. The highest prevalence of CE cases in women and men occurred in 2013 (35.41%) and 2013 , 2017 (34%) , respectively . The affected organ was liver in 59.2% patients.

Discussion and Conclusion: According to high prevalence of cystic Echinococcosis and its financial burden, control programs should be essential for decreasing the burden of disease in this region.

Keywords: Cystic Echinococcosis, human, Hamedan

Situation of Malaria in Iran, 2008-2017

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Background: Malaria has been prevalent in Iran for many years and is now endemic in southern Iran. Control programs have reduced the number of people with Malaria. The aim of this study was to evaluate the status of malaria in Iran over the past 10 years as well as Iran's position in the global program for the control and eradication of Malaria.

Material: Data of Malaria were obtained from WHO annual reports. Also, related articles were studied and the required information was extracted and entered into the SPSS software. Annual Parasite Incidence (API), the most prevalent species of *Plasmodium*, the rate of death, control tools and the first line treatment was determined over these years.

Results: The results of our study showed the pattern of Malaria outbreaks in Iran is decreasing. So that in 2008, API was 0.16, while in 2017 it reached 0.011. In 2017, 93.83% of all reported malaria cases were imported. *Plasmodium vivax* has been the main cause of malaria in Iran for all years. In 2017, only 9% of the reported cases of malaria were due to *Plasmodium falciparum*. The main drugs to treat Malaria were Chloroquine, Primaquine and Artemisinin. Indoor residual spraying (IRS) and insecticide-treated mosquito nets (ITNs) are the most important malaria control strategies in Iran. Iran was in the pre-elimination and elimination phases in 2008 and 2017 respectively.

Conclusion: Iran has been successful in control of malaria to date, and it is hoped that it will receive certification of Malaria elimination in 2025.

Keywords: Malaria, *Plasmodium*, Iran, WHO, API

Genetic Identification of *Echinococcus granulosus* Isolates in Stray Dogs and Golden Jackals in Ilam Province, Western Iran

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Background and aim: Hydatidosis is one of the main zoonotic parasitic diseases with worldwide distribution. *Echinococcus* spp. As an agent of disease shows a high genetic diversity and includes a complex of different genotypes. So this study is designed to investigate *Echinococcus granulosus* diversity in stray dogs and golden jackals using ribosomal ITS1 marker in Ilam province, western Iran.

Materials and methods: Parasites were collected from April 2018 to June 2019. After DNA extraction, rDNA-ITS1 gene was amplified and PCR products digested using AluI, RsaI, and HpaII enzymes. To confirm the results of PCR-RFLP, the sequence of PCR products were determined. The data were analyzed using MEGA6 software.

Results: 20 out of all 120 isolates (65 dogs & 55 jackals) were suffering from *Echinococcus granulosus*. The PCR-RFLP results with the enzyme AluI, RsaI, and HpaII obtained with patterns 200, 800, 400, 600, 300 and 700 bp in dogs and jackals respectively.

Conclusion: All isolates showed similar genotypes. Sequencing and enzymes digestion results showed that there is at least one genotype of the parasite that belongs to *E. granulosus* complex sensu stricto (G1-G3).

Keywords: Genetic diversity, *Echinococcus*, rDNA-ITS1, PCR-RFLP, Iran

Determination of infection rate of Nosemosis in Kohgiluyeh and Boyer Ahmad province

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Introduction & Objectives: The conditions of the Kohgiluyeh and Boyerahmad province and the presence of special plants such as Ziziphus in tropical regions also fragrant plants of the Dena mountain in cold regions have made the honey of this province a special characteristic and flavor. More than 700 tons of honey is produced annually in the province, which is a product of between 80 and 100 thousand bee colonies. Due to the phenomenon of drought and dust in recent years, as well as the presence of ectoparasites such as mites in the apiaries of the province, the production has decreased by almost 50% in comparison with previous years. The aim of this study is determine the rate of infection of Nosemosis in Kohgiluyeh and Boyer Ahmad province.

Materials & Methods: This study was conducted from December 2016 to November 2017 for one year in 182 apiaries from 8 cities of the province to investigate the protozoan disease of honeybee: "Nosemosis". Of the total number of 96,258 hives, 5% of the hives were selected from each apiary, and after being transferred to the laboratory under freezing conditions, their abdominal rings were removed with scalpel. Then they were poured into a Crucible and added 2-3 ml of distilled water per bee. After bruising the abdominal rings, a wet slurry obtained from the resulting solution and investigated by microscopic examination.

Results: In this study, the rate of infection of honeybees was higher in spring (45/9% and lower in autumn 13/2%).

Conclusion: This infection rate can be due to weather conditions (temperature & humidity).

Keywords: Honeybee, Nosema disease, Kohgiluyeh and Boyerahmad

Prevalence of *Theileria* infection in livestock of Baneh city in cold seasons of year (Seasons in which the activity of carrier ticks reduces)

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Theileria infection is caused by a type of blood intracellular protozoan. Activity of the carrier ticks reduces in cold seasons and their reproduction usually stops. Current article aims to analyze prevalence of theileria in unusual seasons.

Between October 2019 and April 2019, 750 peripheral blood samples were taken from livestock referring to Primer polyclinic. Referral cases for non-infectious reasons were excluded from the scope of the study. Also jugular blood samples were taken from cases with clinical signs of anemia. Smears were microscopically studied using Giemsa staining.

From 750 blood samples, 105 (~14%) were positive in terms of theileriosis disease. Of the 105 positive cases, 23 were cows, 30 were calves under 2 months, 19 were sheep and 28 were goats. In 8% of them, Koch's blue body was observed in the peripheral blood in which the most frequent was in goats. Also in about 4% of the cases, no parasites were observed directly but based on clinical signs and other blood parameters, Theileriosis diagnosis was performed and the response to treatment was also followed up. In these cases, the highest incidence referred to calves under 2 months which was arisen from maternal transmission. According to the reduction in ticks activity in the cold seasons, the incidence of theileriosis disease in these seasons is a significant issue.

Climate change seems to have increased environmental resistance and the range of activity in the mites. Also, the resistance to treatment, resistance to drug dosage and failure to complete the treatment process have a significant effect on the resistance and the disease's re-emergence in which symptoms such as turning sickness as a result of a recurrence of the disease may be observed. It is recommended that clinicians be more careful about the illness in unusual seasons. And also using complementary experiments such as PCV and lymph node sampling in addition to blood smears.

Keywords: *Theileria*, Theileriosis, livestock, Baneh

Prevalence and long term trend of hydatidosis, fascioliasis and dicrocoeliasis in slaughtered sheep and goats in North-West of Iran during 2001-2018

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Background and objectives: Hydatidosis, fascioliasis and dicrocoeliasis are zoonotic diseases that are responsible for great economic loss and getting public health at risk. This study aimed to determine the prevalence and 18-year trend of these infections in the slaughtered sheep and goats in West Azerbaijan province during the years 2001-2018.

Materials and Methods: In this cross-sectional study, we used information on reports of meat inspection in slaughterhouses of the province's veterinary organization to determine the prevalence and 18-year trend of the hydatidosis, fascioliasis and dicrocoeliasis in sheep and goats.

Results: A total of 1075744 sheep and goats were slaughtered during this period. The 18 year prevalence of hydatidosis in liver (HC-H) and in lung (HC-L), fascioliasis, dicrocoeliasis, and fascioliasis and dicrocoeliasis (F&D) were 5%, 11%, 3%, 7%, and 1% respectively. The overall 18-year trends for HC-H, HC-L, and dicrocoeliasis were upward; but the trends for fascioliasis, and F&D were downward and linear. The lowest rates of HC-H and HC-L were observed in the year 2002 (2.88%, 5%, respectively) and the highest rate of HC-L (18.95%) and HC-H (9.74) were seen in 2015 and 2016. The lowest and highest rate of dicrocoeliasis was seen in 2002 (3.84%) and 2016 (15%). The lowest rate of fascioliasis, and F&D was seen in 2018 (1.1% and 0.2%, respectively).

Conclusion: The prevalence of hydatidosis and dicrocoeliasis has been increased in recent years, which shows the need to improve the prevention programs in animals, as well as careful monitoring of sheep and goat bodies in slaughterhouses.

Keywords: hydatidosis, fascioliasis, dicrocoeliasis, West Azerbaijan

Agrostemma githago increases caspase 3/7 activity in *Leishmania major* promastigotes

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Abstract

Introduction and objectives: Acquired resistance to anti-Leishmanial drugs are increasing worldwide. Anti-Leishmanial effect of *Agrostemma githago* seed extract on *Leishmania major* promastigotes has already been shown in our laboratory. This study was conducted to assess the induction of apoptosis in *Leishmania major* promastigotes after treatment with *Agrostemma githago* seed extract.

Methods: The activity of caspase 3/7 as the key executioners of apoptosis were measured using the Caspase-Glo 3/7 Assay kit. Following treatment with EC₅₀ dose of *Agrostemma githago* (0.4 mg/ml), 1:1 ratio of Caspase-Glo 3/7 reagent and *Leishmania major* promastigotes suspension containing 5×10^6 cells were added into white-walled 96 well plates and incubated at room temperature for 3 hours. The cleavage of tetrapeptide sequence DEVD by caspase enzymes was measured at 570 nm emission using plate-reading luminometer (Synergy HT, BioTek). Data are reported as the relative caspase activity in comparison to control group.

Results: Our data showed that the activity of caspase 3 and 7 were increased in *Agrostemma githago* received group in comparison to untreated control.

Discussion and Conclusion: Aqueous extract of *Agrostemma githago* seed induced apoptosis in *Leishmania major* promastigotes via increasing caspases 3 and 7 enzymes activity.

Keywords: *Leishmania major*, *Agrostemma githago*, Apoptosis, Caspase 3/7

Microscopical study of *Hepatozoon* spp. in shelterdogs of Hamedan, Kermanshah and Khuzestan Provinces of Iran

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Background and objectives: Hepatozoonosis caused by *Hepatozoon canis* and *Hepatozoon americanus* a protozoan parasite infecting carnivores worldwide. *Hepatozoon canis* is endemic in the Middle-East. Dogs usually get infected by ingestion of *Rhipicephalus sanguineus sensu lato* ticks which contain oocysts of the parasite. Although *H. canis* infection is usually subclinical in young animals and in the case of high parasitemia, the signs may include anaemia, fever, poor appetite, weight loss and enlarged lymph nodes. The diagnosis is often based on the microscopical findings of a stained blood smear. Aim of this study was to examine blood of rescued dogs in shelters in Hamedan, Kermanshah and Khuzestan provinces for the presence of this parasite.

Methodology: From October 2018 to June 2019, a total of 235 EDTA blood samples (100 from Hamedan, 55 from Kermanshah and 80 from Ahvaz) were taken from dogs kept in shelters and breeding centers. Giemsa stained blood smears were examined microscopically.

Results: Gamonts of *Hepatozoon* spp. were observed in 12.34% (29/235) of the neutrophils in blood smears. Infection of dogs was 45.45% (25/55) in Kermanshah and 4% (4/100) in Hamedan. No infected dog was detected in Ahvaz. Co-infection with *Hepatozoon* and *Cercopithifilaria bairdii*, a dermal filaroid of dogs, was detected in 6 dogs (20.69%).

Discussion and Conclusion: Canine hepatozoonosis was previously reported from Khorasan (infection rate=1.57% and 8% in two studies), Ardabil (infection rate= 23.07%) and Tehran (infection rate= 22.06%). Our study provides information about *H. canis* infection in dogs in west and south-west of Iran. This parasite has been also detected in a Persian leopard (*Panthera pardus ciscaucasica*) suggesting that wild carnivores have a role in the epidemiology of disease in the country.

Keywords: *Hepatozoon canis*, Hepatozoonosis, Canine, Vector-borne

The effect of ethanolic and aqueous wild rue extract on Galactic Trichomonas in laboratory environment

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Abstract

In this study, the effect of Ethanol and Aqueous wild rue extract (with scientific name of *peganumharmala*) on Protozoan parasite (*Trichomonas gallinae*) was evaluated.

This parasite was cultivated in a TYIS-33 culture medium in Pasteur Institute of Karaj, until their number reached 1×10^5 and the temperature in the incubator was 36 centigrade.

After cultivating the parasite, the samples were kept in an incubator for 48 hours in 36 centigrade temperature.

The extract was diluted in a solvent with base of 100mg/ml. we added the instilled water to dilute the extract with condensation of 50, 25, 12.5, 6.25 and 3.125mg/ml.

To this end we used 96 sink micro plates including 8 rows of twelve with volume of 300 λ . In the sinks the micro plates of the extract with defined condensation that contained the parasite was poured. In each there were sink 200 λ include 100 λ with defined condensation and 100 λ the culture environment contained *Trichomonas gallinae* with required number and they were kept in anaerobic conditions. In the sink 1 or the subject sink there was 100 λ distilled water, in the sink 2 or the control sink there was 100 λ Metronidazole at a concentration of 25 $\mu\text{g} / \text{ml}$, in the sink 3 there was ethanol or aqueous extract with concentration of 100mg/ml and in sinks of 4 to 8 the condensation of the extract were 50, 25, 12.5, 6.25, 3.25, respectively. Then the 100 λ cultivated parasite was added to the sink and were incubated in 36 centigrade degree. At the end, during the incubation, in 2,6,12,24 and 48 hours interval 20 λ removed from each sink and 20 λ Trepan blue was added and the number of alive and dead was numbered using a hemositometer lam.

Keywords: wild rue, *Trichomonasgalyne*, Ethanol & aqueous extract, Metronidazole

***Toxoplasma gondii*, a forgotten parasite in northern Iran with remarkable exposure (a cross sectional study)**

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Background& Objectives: Toxoplasmosis is a worldwide disease caused by an obligate intracellular protozoan, *Toxoplasma gondii*. Approximately 30 % of human population worldwide is chronically infected with *T. gondii*. Immunocompetent individuals infected with *T. gondii* are typically asymptomatic and maintain this infection for life but in immunocompromised individuals is dangerous. The aim of this study was to determinate the anti-*Toxoplasma* antibodies (IgG and IgM) in rural population of Qaemshahr, North of Iran in 2019.

Materials and Methods: Sera of 370 individuals referred to Rural Health Centers Laboratories in Qaemshahr city in Mazandaran province of Iran during March to April 2019 were collected and examined for anti-*Toxoplasma* (IgG and IgM) antibodies using ELISA kits (Pishtazteb, Iran). The results were analyzed using Chi-square test.

Results: 307 (82.97%) out of 370 sera were positive, 4 (1.08%) were borderline and 59 (15.94%) were negative for anti-*Toxoplasma* IgG. No samples were positive for anti-*Toxoplasma* IgM. No significant association was observed between seroprevalence of Toxoplasmosis and age, gender, consumption of raw or undercooked meat. There was significant correlation between consumption of raw vegetables, owning cat and positive anti-*Toxoplasma* IgG ($P < 0.05$).

Conclusions: Prevalence of Toxoplasmosis among rural people of Qaemshahr city in north of Iran was much higher than those previously reported from other areas of Iran. Efforts should be focused on public health education to reduce the Toxoplasmosis transmission chance especially in high risk groups in this region.

Keywords: Toxoplasmosis; *Toxoplasma gondii*; Rural Health Centers; ELISA

The seroprevalence of Toxocariasis in hypereosinophilia and normal people referred to Arash Hospital

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Background: Hypereosinophilia is one of the common signs of some parasitic infections. Among helminthic infections, moderate to severe eosinophilia can be seen in Toxocariasis as a pathophysiological response to worm infection. The aim of this study was to determine the *Toxocara* seropositive rate among hypereosinophilia and normal people referred to Arash Hospital in Tehran.

Materials and methods: This cross-sectional study was done during Feb 2018 to Mar 2019. Serum samples of 100 patients with Hypereosinophilia (>10%) and also 100 normal persons were collected. Demographic information of participants was collected in a questionnaire. Anti-*Toxocara* antibodies in the serum of the persons were examined by enzyme-linked immunosorbent assay (ELISA) test.

Results: In Hypereosinophilic patients, 68 (68%) were male and 32 (32%) were female. Range of eosinophilia was between 10-50%. Anti-*Toxocara* antibodies were detected in serum of 2 (2%) of patients. Of 100 normal persons, 65 cases (65%) were male and 35 (35%) were female and anti-*Toxocara* antibodies were detected in serum of 3 (3%) persons. No significant correlation was found between hypereosinophilia and the presence of anti-*Toxocara* antibodies ($P > 0.05$).

Conclusion: Hypereosinophilia occur for various reasons, including Toxocariasis. In fact, hypereosinophilia is not a sufficient reason for the diagnosis of Toxocariasis and this sign, along with other factors, helps to diagnosis of Toxocariasis.

Keywords: Hypereosinophilia, Toxocariasis, Seroprevalence, Elisa

Anti Leishmanial and cytotoxic effects of various extracts of *Capparis spinosa*.

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Background: Today, one of the most important parasitic infections in the world is cutaneous Leishmaniasis (CL) which human being infected by the protozoa of the genus *Leishmania*. Today, the common and selective drugs for the treatment of CL are antimonials compounds which have some limitations in use. This study aims to investigate the antileishmanial and cytotoxic effects of various extracts of *C. spinosa* on in vitro model.

Methods: Here we evaluated the in vitro anti Leishmanial and cytotoxic activity of *C. spinosa* extracts on *Leishmania tropica* promastigote by colorimetric cell viability (MTT) assay. Moreover, cytotoxic effects of extracts on J774-A1 cells were also measured by colorimetric MTT assay.

Results: The findings indicated that the methanolic and aqueous extracts of *C. spinosa* were had high potency to inhibit the growth of *L. tropica* promastigotes with IC₅₀ (50 % inhibitory concentrations) values 28.5 and 44.6 µg/ml, respectively. Based on the obtained results, *C. spinosa* extracts did not display considerable cytotoxicity on J774-A1 macrophage cells.

Conclusion: The obtained findings exhibited remarkable anti Leishmanial effects *C. spinosa* extracts on *L. tropica*; indicating the ability of *C. spinosa* as a natural cause for to create a new anti Leishmanial drug. Nevertheless, supplementary investigations will be obligatory to reach these findings, especially in human subjects.

Keywords: *Leishmania tropica*, in vitro, herbal medicines, promastigote, macrophage

Analysis of B1 and ROP8 gene regions for designing High-resolution melting technique and separating different types of *Toxoplasma gondii*

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Differentiation and characterization of *Toxoplasma gondii* types plays an important role in the health management and epidemiology of Toxoplasmosis. Correct and accurate recognition of the various genetic regions of the parasite is important to select an appropriate region for designing a High-Resolution Melting analysis (HRM). Designing and optimizing HRM analysis for isolating of different types of *Toxoplasma gondii* were main objective of this research. A total of 96 DNA samples of muscle tissue of livestock (cattle, goats and sheep) and poultry brain with three standard strains of *Toxoplasma gondii* (RH, PRU and VEG strains) were prepared and analyzed. Two gene regions of *B1* and *ROP8* were targeted. Nested-qPCR-HRM specific new primers were designed and synthesized. Bioinformatics analyzes were employed to predict the temperature resolution of DNA between different types. To optimize the reaction, 30 positive samples of different types were sequenced and compared with HRM results.

The results of amplified gene regions with innovated primers showed that *B1* gene were able to separate type 1 ($T_m = 84.8^\circ\text{C}$) from two other types ($T_m = 84.6^\circ\text{C}$). Also, the *ROP8* gene was able to separate type 2 with the average melting temperature of 84.5°C from 1 and 3 types ($T_m = 84.12^\circ\text{C}$).

According to the results, in silico predictions were completely consistent with the results of the temperature melting behavior of DNA. Also, comparing of temperature variations of two genes with the results of PCR-Sequencing method shows that the *ROP8* gene was better validated than *B1* gene for optimizing HRM reaction.

Study of *Eimeria* species diversity in native chickens of Qaemshahr suburb

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Abstract

Eimeria infection in native chickens is common throughout the world and Iran, and causes remarkable infection in broiler chickens of the same geographic region. This study was aimed to determine prevalence and diversity of *Eimeria* species in native chickens of suburban villages of Qaemshahr municipality, Iran. A total of 220 indigenous chickens from three geographic regions were sampled using cluster sampling method from summer 2017 to summer 2018. The intensity of infection was determined on the basis of *Eimeria* oocyst per gram of feces (OPG) using Clayton-Lane and McMaster methods. *Eimeria* species diversity and prevalence were also determined by using oocyst sporulation in 2% potassium dichromate. Of all examined native chickens, 35 (15.9%) chickens were infected with *Eimeria* species. Of all examined native chickens, four *Eimeria* species were identified, i.e. *E. necatrix* (8.5%), *E. tenella* (11.42%), *E. maxima* (31.42%) and *E. acervulina* (42.85%). Mixed infections with 2 (5.71%), *Eimeria* species were also identified. The results of the current study elucidated that different *Eimeria* species were prevalent in native chickens of the suburban villages of Qaemshahr municipality, which could be important in epidemiology of infection spreading to the neighboring broiler and broiler breeder farms of the region.

Keywords: *Eimeria*, species diversity, native chickens, Qaemshahr

Report of two separate cases of two headed protoscolex revealed by Phase contrast microscopy from southern Iran

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During a study of protoscolicidal agents on sheep protoscoleces and investigations on their microscopic changes using Phase contrast microscopy, we observed two separate cases of two headed invaginated and evaginated protoscoleces. In evaginated case, two heads were attached to a unique bigger size of protoscolex body. Morphological observations showed its dimension around two times of usual protoscolex. There was no space between the bodies, hence one fused body was observed which was clearly shown by Phase contrast microscopy. Each head possessed two rows of hooks each. The hooks in two headed invaginated case were measured 32 and 34, hence in total 66 hooks were observed in invaginated two headed protoscolex. All parts were clearly visible and photographed by Phase contrast microscopy. Specific parts of two headed protoscolex including suckers, hooks and also calcareous corpuscles were measured. Calcareous corpuscles were measured in evaginated two headed as a total of 120 which is higher than usual. Using micrometry all parts of the two headed protoscolex specially hooks were measured and appropriate photos were photographed showing all aspects of its morphology including tegument, hooks, two heads etc. with different magnification of Phase contrast microscopy.

Keywords: Hydatid cyst, two headed protoscolex, Abnormal morphologic

The study of soil contamination by *Toxocara* spp. eggs in different areas of Chaharmahal va Bakhtiari Province, Iran

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Abstract:

Background and aims: Toxocariasis caused by *Toxocara* spp. is a zoonotic infection with a global distribution and is considered as an important health problem in human. Soil is considered as the main source of transmission of *Toxocara* infection to susceptible hosts. In this study, we investigated the existence of *Toxocara* spp. ova in rural and urban public areas of Chaharmahal va Bakhtiari Province.

Methods: From May to September 2017, soil samples from 74 public places were collected from nine counties of Chaharmahal va Bakhtiari province. The samples were examined for *Toxocara* eggs by the sucrose flotation method and direct smear.

Results: The contamination rate of soil samples with *Toxocara* spp. ova was varied from 0-18.1 % in different counties. Overall, out of 180 soil samples examined, 9 (5%) samples were found positive for *Toxocara* spp. ova. Of nine districts investigated, *Toxocara* spp. ova was found in four counties. The highest rate of soil contamination was found in Farsan county with 18.1%.

Discussion and Conclusion: This was the first study of *Toxocara* spp. soil contaminated in public rural and urban areas of Chaharmahal and Bakhtiari province. According to the result of this study, the rate of soil contamination in this region was lower compared with other parts of Iran. The further study is required to determine factors that may involve in distribution of *Toxocara* spp. in different areas of this province.

Keywords: *Toxocara*, Flotation, Soil, Chaharmahal va Bakhtiari, Iran

Prevalence of *Fasciola Hepatica* in Slaughtered cattles in Maku Free Zone

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Background: Fasciolysis refers to an infection caused by human and animal infections with feces of the genus *Fasciola* (*Fasciola hepatica* and *Fasciola gigantica*). *Fasciola* parasites are tertiary-worm-worms that are the natural parasite of ruminating animals, including sheep, goat, cattle, buffalo, pigs, camels.

Methods: This study was carried out descriptively and prospectively concerning the slaughter chart of the years 1397 and 1398 available in maku industrial slaughterhouse and the veterinary office of the maku. The information present in the charts were transferred to the tables designed according to desired variables such as the kind and the number of the slaughtered domestic animals, the kind of the parasite, the number of the infected domestic animals, month, season and the year.

Results: the prevalence of *Fasciola hepatica* was In this study Of the 1200 cattles slaughtered in the Maku slaughterhouse, 80 vertebrae had liver infected with *Fasciola hepatica*. Pollution in winter was 13% more than in other seasons.

Keywords: *Fasciola hepatica*, Infection, Slaughterhouse

Study the effect mix of *Peganum harmala* & Lavender plantes on the death of *Pediculus humanus*

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AbstractBackground : Lice is an external parasite that dates back to prehistoric times. Lice feeds on human blood and its saliva contains anticoagulants and prevents blood clots and causes severe itching. A 2011 study showed that lavender oil helps prevent head lice growth and can even cure it.

AbstractBackground Methods: In this study, 110 infected people were examined. The age group was between 9 years old and 40 years old. The spin extract and its combination with lavender were used.

Results: The dilutions used were 100, 50, 25, 10 percent, respectively. The death rate in adult lice at a concentration of 100% over a three-day use period, was 93%. This study shows Herbal compounds can be replaced by chemical compounds in the treatment of lice.

Keywords : Concentration- Treatment- *Peganum harmala*- *Pediculus humanus*

Human hydatidosis based on hospital records in Semnan city, Iran: A 12years survey (2006-2017)

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Background& Objectives: Hydatidosis is one of the most common zoonotic parasitic diseases which is endemic in many parts of the world, including Iran. The disease is developed in humans after ingestion of *Echinococcus granulosus* eggs producing hydatid cyst/s in every organ more common in the liver and lungs. To evaluate the status of human hydatidosis in this region, a retrospective study was conducted in Semnan city hospitals from 2006-2017.

Methods: The descriptive-analytical study included 87 patients at public hospitals in Semnan city. The information including patient's records, demographic data, clinical features, laboratory findings, diagnostic criteria, surgical approaches, and outcome were collected from 3 general hospitals and analyzed by descriptive statistics.

Results: Out of 87 patients, a total of forty eight males (55.2%) and 39 females (44.8%) were recorded, respectively. A total of 28 (32.6%) of the patients were in the age of 16-30 years old, showing the highest infected age group. Liver with 47 (54%), followed by lungs 27 (31%) were the highest infected organs. In five (5.7%) cases, cysts were found in both liver and lungs and just in 8 (9.2%) cases, the cysts were found in other organs (Spleen, Muscle, Pelvis, Abdominal cavity). There was no statistical significant association between sex groups ($P = 0.335$); however, a significant association was found between age group, residency and the organ involved ($P = 0.000$).

Conclusions: Our study emphasized the importance of hydatid cyst in Semnan. More extensive epidemiological investigations and monitoring are necessary to determine the prevalence, economic impact and risk factors for the disease control.

Keywords: Human Hydatidosis, Hospital Records, Semnan

Proteomic characterization of hydatid cyst fluid: Comparison of different methods to achieve better resolution

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Background: Cystic echinococcosis/hydatidosis, caused by *Echinococcus granulosus* metacestode, is one of the most deleterious helminthic diseases of humans and animals. High protein content of hydatid cyst fluid (HCF) which is a host-parasite interaction result, has a major role in the disease. Identification and characterization of proteins of HCF might help to find new candidates for the immunodiagnostic and vaccines developments. However, due to large amount of host serum proteins, especially albumin and globulins and non-protein content, may lead to resolution problems and proper identification of parasite proteins especially in proteomics studies. The purpose of the present study is to compare different methods to achieve better resolution in proteomic characterization of HCF.

Materials and Methods: Hydatid cyst fluid (HCF) were obtained from infected sheep liver hydatid cyst. HCF was aseptically aspirated from cysts and centrifuged at 10,000 g, for 15 minutes at -4° C for sedimentation of protoscolerces and debris. To purify the HCF, dialysis, Amicon ultra centrifugal filter and exchange buffer were applied to the HCF.

A total of 5 methods including TCA/Acetone, Acetone, lyophilization, lyophilization with TCA/Acetone and clean up kit were used. The results were compared to achieve the best or superior method for better resolution in protein extraction.

Results: Using 2DE of TCA/Acetone, Acetone and lyophilization method showed a lot of smear in background and prevented proper identification of parasite proteins, lyophilization with TCA/Acetone showed a better resolution of background, smears and impurities were greatly reduced and clean up kit showed the best result and almost all impurities were removed. Application of five methods using two dimensional electrophoresis (2DE) showed that the lyophilization with TCA/Acetone and using clean up kit will be more appropriate resulting better resolution.

Conclusion: Comparison of the two dimensional electrophoresis (2DE) showed that the best methods for proteomic characterization of hydatid cyst fluid for 2DE were lyophilization with TCA/Acetone and using clean up kit.

***Leishmania infantum* infection of *Phlebotomu salexandri*, a probable vector of zoonotic visceral leishmaniasis in Khuzestan Province, Iran**

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Leishmaniasis is an important vector-borne disease in Khuzestan Province, west of Iran in the last decade. Based on the reports of Center for Disease Control (CDC) of Iran, Zoonotic Cutaneous Leishmaniasis (ZCL) is prevalent in this province but Zoonotic Visceral Leishmaniasis (ZVL) is not endemic in the region. This study was conducted for determining the *Leishmania* infection of *Phlebotomus alexandri* in some selected areas of the province. Sand fly specimens were collected by sticky and light traps and were identified using valid morphological keys. The specimens were examined using nested-PCR and DNA sequencing techniques for detection and identification of *Leishmania* parasite. The results showed that *P. alexandri* was infected by *Leishmania major* and *Leishmania infantum*, the causative agents of ZCL and ZVL respectively. The mentioned sand fly is incriminated as suspected vector for ZVL in Fars province. As infection to *L. infantum* in this species was found in some parts of northern area of Khuzestan Province and WHO reports state that ZVL is endemic in eastern Iraq provinces, it seems that Khuzestan could be a potential focus for ZVL.

Keywords: *Phlebotomus alexandri*, *Leishmania*, Leishmaniasis, Khuzestan, Iran

Therapeutic Effects of Alcoholic Extract of *Lepidium latifolium* on *Pediculus humanus*

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Objectives: However, head lice are a major public health problem in both developed and developing countries. It causes itching and inflammation of the scalp and neck, enormous pruritus, allergic reaction, exudations, lymphadenopathy, and eczema. Most synthetic chemical insecticides are harmful and toxic for human health. Consequently, research for safer and more effective alternatives to protect children from harmful effects of synthetic chemical insecticides is needed to be done.

Materials and methods: The leaves of the plant *L. latifolium* were isolated and dried under shade. The alcoholic extract was prepared and applied as a lotion. Concentrations of 0.12, 0.25, 0.50 and 0.75 ml/cm² from herbal extract were examined. The lice mortalities were recorded at 5, 15, 30, 45 and 60 min.

Results: Our data showed that this herbal extract formulation of a native plant in Iran is suitable to be used as pediculicides with no side-effect for children. There are some reports of resistance to topical and systemic pediculicide and different class of pediculicide should be used for treatment.

Discussion and conclusions: Head lice must be controlled as a serious disease at an early stage. Also, due to certain kinds of drug resistance, available shampoos may not be effective in this regard. The present study showed that *Lepidiumlatifolium*can well control the disease without side effects.

Keywords: Pediculosis, *Lepidium latifolium*, Herbal extract, Infestation

An Experimental Study on Protozoan Infections (*Giardia*, *Cryptosporidium*, *Entamoeba* and *Isoapora*) in Stray Dogs in Ilam City West of Iran

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Background and aim: Protozoa are important parasites that caused diarrhea in human and animals.

Materials and method: In this experimental study, fecal samples were collected from the small intestine of the 82 stray dogs from different locations of Ilam city and suburbs road accidentally, from April 2018 to June 2019. Within the period of one year, their intestinal specimens were checked for protozoan infections. *Giardia* and *Entamoeba* were concentrated by using the formalin ether sedimentation method followed by the trichrome and iodine staining technique. *Cryptosporidium* and *Isoapora* oocysts were concentrated by using the formalin ether and shifter sedimentation method followed by the modified Ziehl-Neelsen staining technique respectively.

Results: Totally of 82 stray dogs, protozoan infections were detected from feces of 19 dogs (23.17%) that *Giardia* infection was detected from feces of 11 dogs (13.41%), *Isoapora* 4 (4.78%), *Cryptosporidium* oocysts were detected from feces of 3 dogs (3.65%). In the present study observed to *Entamoeba* only in 1 case (1.21%).

Discussion and Conclusions: No statistically significant differences in prevalence of protozoan parasites occurred between female (25 %) and male (21.05 %) stray dogs ($P > 0/05$). No statistically significant differences in prevalence occurred between $1 \geq 0$ and $0 \geq 1$ stray dogs ($P > 0/05$). So that stray dogs of Ilam city can cause infection of human water and food sources.

Keywords: *Cryptosporidium*, *Giardia*, *Entamoeba*, *Isoapora*, Stray dogs, Ilam

A case report of ectoparasites of wild pig (*Sus scrofa*) from Abdanan region, Ilam province, west of Iran

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Introduction and Objectives: The Eurasian wild pig (*Sus scrofa*), is found throughout tropical and temperate regions of Iran and serious threat to agricultural farms. Ectoparasites, in addition to causing lesions, act as important carriers for some pathogens. The aim of the work was investigation on ectoparasites of wild pig from west of Iran.

Methods: A 2-month old wild piglet were captured following the wild pig herd attack to the wheat farms in Abdanan region of Ilam province, west of Iran.

Nearly all the ectoparasites of the animal were collected by parasitological methods, preserved them in 70% Ethanol and transferred to the laboratory of parasitology in Shahid Chamran University. they were counted, some of them cleared in 10% KOH solution and then identified morphologically using valid diagnostic keys.

Results: Identification of the collected parasites showed 5 ticks from back of the animal ears and 59 lice (15 males, 27 females and 17 nymphs or larvae) from abdominal, perineal and neck areas. The all lice were belonged to a species, *Haematopinus suis*. The ticks were identified as species of *Hyalomma anatolicum excavatum* (1 female and 1 male) and *Hyalomma anatolicum anatolicum* (3 males).

Discussion and conclusion: wild pigs are able to carry important viral and bacterial diseases and susceptible to several protozoal and helminth parasites, including some that pose a zoonotic risk to people. These findings suggest wild pigs may assist to expansion of some tick species, therefore, they could be used as a source for detecting and monitoring of tick species.

Keywords: wild pig, ectoparasite, Iran

Case report: First report infection with *Trichostrongylus retortaeformis* in a European hare (*Lepuseuropaeus*) in Shahr-e-Kord city, Chaharmahal o Bakhtiyari Province, Iran

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Back ground: *Trichostrongylus retortaeformis* is one of the hare intestinal nematodes that penetrate the mucosa and causing desquamation and, in heavy infections, inflammation of the intestine with excess mucous exudate. Examination of wildlife animals disease for their free-living and their role in the transmission of the disease is important. The aim of this report was examining wildlife animals intestinal parasitic disease that they killed by a road accident. Infection with *Trichostrongylus retortaeformis* reported for the first time by Garedaghi and et.al (Garedaghi Y., Tarrahi A., Khodkar A. 2010) in Iran, Ardabil city. This parasite has been reported by us for the first time in Chaharmahal o Bakhtiyari and there is not any history by infection with this parasite in recent Province.

Methods: After referring one adult male European hare to parasitology lab that killed by a road accident, we checked hare feces for intestinal parasitic disease using Mc master method .

Results: Strongle shape eggs seen under a light microscope by x40 magnify and for decisive diagnosis, we did feces culture and after counting intestinal cells of the third larval stage, infection with *Trichostrongylus retortaeformis* have been diagnosed.

Conclusion: The result of this report indicated that infection of wildlife animals to various parasites such as *Trichostrongylus retortaeformis* in European hare exist and it is important because they are free and there is not any control on their disease, for example, their parasitic disease. Infection with this parasite in hare has been reported in Chaharmahal o Bakhtiyari for the first time.

Keywords: *Trichostrongylus retortaeformis*, European hare, Shahr-e-Kord, Iran

Comparison the clinical and parasitological findings of Malignant Ovine Theileriosis in sheep and goat during an experimental study

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Introduction and goals: Malignant Ovine Theileriosis (MOT) is one of the most important tick-borne disease caused by *Theileria lestoquardi* among small ruminants. It is distributed from Mediterranean region to China. According to many references, goats are resistant to malignant theileriosis. The study was carried out to compare the clinical signs and parasitological findings in sheep and goats after being experimentally infected by *T. lestoquardi*.

Material and methods: In an experimental study, *T. lestoquardi* infection, was transmitted to a goat and sheep by infected *Hyalomma anatolicum* ticks. The hosts were clinically examined. Blood and lymph nodes smears were collected during the study.

Results: The sheep and the goat showed clinical signs including fever, pre-scapular lymph nodes enlargement, anemia and mucosal membrane pallor. After 12 days post infection (PI), body temperature in the goat rose to 40.5°C while the temperature for the sheep was 42°C. Pre-scapular lymph nodes enlargement was notable in the sheep than the goat but the goat showed earlier and more severe mucosal membrane pallor during study. The parasitemia was 2% in the infected goat and 2.8% in the sheep on day 12 PI. The infected goat showed better response to Buparvaquone treatment compare to the sheep so that after 3 months post infection, it was difficult to find piroplasms in the goat blood smear while they were still detectable in blood smear of the infected sheep.

Discussion and conclusion: According to our findings, goats are more resistant to theileriosis than sheep and it seems that goats can reduce the virulence of theileriosis among small ruminants.

Keywords: *Theileria lestoquardi*, Experimental infection, Sheep, Goats

In vitro and ex vivo effects of *Quercus infectoria* extract on hydatid cyst protoscoleces

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Background: Currently, surgery is well-known as the most useful treatments for hydatidosis. Because of leak the cyst contents and dissemination of the protoscoleces during surgery that can cause dangerous risks, surgeons applied a number of chemical agents as protoscolicidal agents. The study aims to evaluate the scolical effects of oak (*Quercus infectoria*) extract on hydatid cyst protoscoleces in vitro and ex vivo.

Methods: Following collection of protoscoleces from sheep livers infected with fertile hydatid cysts; they were treated with the various concentrations of oak extract (250-1000 µg/mL) for 5-40 min in vitro and ex vivo. Finally, the mortality of protoscoleces was assessed by the eosin exclusion test (0.1% eosin staining).

Results: The mean of the mortality of protoscoleces was 100% after 5 min incubation with the concentration of 1000 mg/ml of oak extract. On the other hand, the mean of the mortality of protoscoleces after 10 incubation with the concentration of 500 mg/ml of oak extract was 100%. However such results were not observed in the ex vivo analysis, so that oak extract requiring a further time to display a potent protoscolicidal effects.

Conclusion: The results of this investigation revealed that oak extract has a significant scolical activity on hydatid cyst protoscoleces. However, further study, especially in human and animal subjects are required to reach this conclusion.

Keywords: cystic echinococcosis, *Echinococcus granulosus*, protoscoleces, in vitro, ex vivo, oak

Seroprevalence of *Toxoplasma gondii* antibodies and associated risk factors among children in Lorestan provinces, Iran

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Background: This cross-sectional study aims to determine the prevalence of IgM and IgG anti-*Toxoplasma gondii* antibodies and the associated risk factors among the Children (5–15 yr Old) Referred to health centres of Lorestan province, Iran from August 2016 to March 2017 on 316 children.

Methods: To determine the prevalence of IgM and IgG anti-*Toxoplasma gondii* antibodies, all the serum samples were tested using the commercially available ELISA kit (Dia.Pro, Milano, Italy). Analyses were carried out according to the manufacturer's instructions. Before collection of blood samples, a questionnaire based on demographic data including age, gender, and education was given to the children. Moreover, possible risk factors, such as animal contact (cat), raw/half-cooked meat consumption (lamb and beef), consumption of raw vegetables and residence were also evaluated.

Results: Out of the 316 children, 31 (9.8%) tested seropositive for anti-*T. gondii* antibodies; 24 (7.6%) children tested seropositive for IgG antibody, 1 (0.3%) tested seropositive for both IgM and IgG, and 6 (1.9%) were positive for IgM antibody alone. The results showed that some risk factors were significantly correlated to *T. gondii* seropositivity included age ($P=0.005$), living in rural areas ($P=0.01$) and consumption of undercooked or cured meat products ($P<0.001$).

Conclusion: The findings of our study demonstrated a considerable seroprevalence of *T. gondii* infection in children in Lorestan province, Iran. Thus, proper strategies must be carried out to prevent and control *T. gondii* infection in children in this region.

Keywords: *Toxoplasma gondii*, Seroprevalence, ELISA, *Toxoplasma gondii* antibodies

Determined the species/strains of the causative agents of cutaneous Leishmaniasis in DashtAzadegan using PCR-RFLP during 2016-2019

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Back ground: As a neglected disease, cutaneous Leishmaniasis (CL) renders considerable incidence rates. Eastern Mediterranean countries like Iran are endemic regarding this infection. Aimed of this study was to assess Determine the dominant species and strain in the in DashtAzadegan.

Methods: we determined the species/strains of the causative agents of CL in DashtAzadegan using PCR-RFLP during 2016-2019. Smear slides from suspected cases referring to health centers were examined microscopically. After DNA extraction from slide materials, amplification of ITS1 fragment was done using LITSR and L5.8S primer pair for *Leishmania* molecular detection. Subsequently, HaeIII (species level), TaqI (strain level), DpnI and HpaII (mutation analysis) digestion was exerted as RFLP method.

Results: Microscopic examination revealed amastigote forms of *Leishmania* in all 80 samples. A 350 bp band was amplified by ITS1-PCR, which confirmed the infection at molecular level. Following HaeIII digestion, 150 and 200 bp fragments were produced indicating *L. major*. Also, TaqI digestion rendered 130 and 200 bp bands suggesting A1 strain. Moreover, no mutations were detected in the genome of identified *L. major* A1 strains by DpnI (140 and 200 bp bands) and HpaII (no digestion) digestion.

Conclusion: The genotypic heterogeneity of *Leishmania* species is of utmost importance for better treatment choices, appropriate diagnosis and preventive measures. Future works in the area should address the parasite strains in alternative vector/reservoir hosts.

Key word: PCR-RFLP, cutaneous Leishmaniasis, DashtAzadegan

Advances in Development of New therapies for Leishmaniasis

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Background: Leishmaniasis is an infectious disease that is caused by intracellular parasites of the genus *Leishmania*. The side effects of anti-parasitic drugs have raised the replacement importance of recombinant proteins with history of antimicrobial properties like *Clostridium septicum* α -toxin. So the aim of this study was to determine the biotechnology based production of bacterial α -toxin to evaluate its role in *Leishmania* killing, as well as facilitating the delivery of glucantime to the macrophage-parasite system *in vitro*.

Materials and methods: The entire coding sequence of α -toxin was designed and inserted to the pET-28a (+) vector. The expression and purification of α -toxin recombinant protein were evaluated by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western blotting techniques. The anti Leishmanial activities of the purified protein plus and without glucantime were examined *in vitro*.

Results: Successful expression of α -toxin as a 48 kDa band was confirmed in SDS-PAGE and western blot methods. Also evaluation of α -toxin IC₅₀ showed strong killing effect of it and glucantime on medium proliferated *Leishmania* promastigotes at lower concentrations compared with glucantime or α -toxin alone.

Conclusion: The results indicated successful recombinant production of α -toxin and suggested recombinant α -toxin plus glucantime can have synergistic effects in eliminating the life of parasite.

Key word: Leishmaniasis, α -toxin, *Clostridium septicum*, Glucantime

Evaluation of parasitic infections in patients referring to Dr. Nemati Laboratory in Urmia city from March 2018 to March 2019

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Background and aim: Parasitic contamination is one of the most important worldwide health problems, especially in developing countries (1). Various types of intestinal parasites can cause contamination in humans and create a wide range of symptoms, depending on different immunological, physiological and social factors. The risk of contamination is high in immunocompromised patients, in patients receiving immunosuppressive drugs and in patients undergoing chemotherapy (2). The aim of this study was to determine the prevalence of parasitic infections in patients referred to Nemati Laboratory in Urmia city in the year of 2018.

Materials and Methods: After referral of patients and delivery of fecal samples, all specimens were examined for the presence of parasites by direct test with physiologic serum and logol. Then, in order to accurately detect the type of parasite, positive specimens were determined by genus and strain using ether formalin concentration and trichrome staining (3).

Results: Of 4814 stool samples (2362 males (49%) and 2452 female samples (51%), 516 samples (10.7%) had parasitic infection, of which 286 cases were male (55%) and 230 cases were female (45%). The prevalence of parasitic infections was as follows: The highest percentage of infection was related to *Chilomastixmesnili* with 15.24%. (202 men with 56% and 158 women with 44% contamination); *Blastocystishominis* with 2.66% (38 men with 60% contamination and 25 women with 40% contamination), *Entamoeba coli* cyst with 2.32% (27 men with 49% contamination and 28 women with 51% contamination); *Giardia lamblia* cyst with 1.05% (14 men with 56% contamination and 11 women with 44% contamination); *Trichomonas hominis* with 0.25% (2 men with 33% contamination and 4 women with 67% contamination); *Giardia lamblia* trophozoite with 0.16%. (2 men and 2 women with a contamination of 50%). The lowest infection rate was related to *Ascarislumbricoides* egg (1 man), *histolytica / dispar amoeba* cyst (1 man) *lodamoebabütschlii* (1 woman) each Which with 0.04 % contamination was.

Conclusion: Parasitic contamination is one of the most important health indicators in today's society. According to the study, the prevalence of *Chilomastixmesnili* was higher than other parasites. By promoting the level of health, chlorination and drinking water filtration, it can be prevented from getting this parasite.

Effect of Photothermal Nanotherapeutics on *Leishmania major* with Gold Nanoparticles

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Leishmaniasis is a parasitic disease caused by *Leishmania* parasites and is one of the biggest health problems of the world. Anti Leishmanial drugs have disadvantages such as toxicity and the recent development of resistance. Previous studies showed that some nanoparticles such as Gold nanoparticles(Au-NPs) have antimicrobial activity. Photo-thermal therapy (PTT) is a minimally-invasive therapy in which photon energy is converted into heat to kill cell. Gold nanoparticles absorb light strongly and convert photon energy into heat quickly and efficiently, thereby making them superior contrast agents for PTT. so, the use of nanoparticles together with infra red (IR) light increases toxic effects of nanoparticles by heat. There is little information on antileishmanial activity of nanoparticles, alone or together with IR. Thus, The aim of this study was to investigate the effects of Au-NPs on *L. major* in vitro. After 24-h exposure to nanoparticles, different biological parameters such as cell viability, proliferation, infectivity, and infection index were investigated under IR and dark conditions. The results showed Au-NP had antileishmanial property, and could inhibit penetration and proliferation of parasite in macrophages. Also, IR rays increased antileishmanial and toxic properties of nanoparticles. Determination of the antileishmanial effects of Au-NPs is very important for the further development of new compounds containing nanoparticles in Leishmaniasis treatment.

Keywords: nanotechnology, Photo-thermal therapy (PTT), Leishmaniasis

Evaluation of acaricidal properties of *Mentha piperita* and *Eucalyptus caesia* Benth essential oils against *Argas persicus*

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Objectives: The genus *Argas persicus* is one of the most important poultry soft ticks in Iran. It is an important vector of *Salmonella pullorum*, *Spirochete gallinarum* and *Pasturella maltocida*, also causes economic loss by sucking blood from host poultry. The objective of this study is evaluation of the acaricidal effect of herbs essential oils (*Mentha piperita* and *Eucalyptus caesia* Benth) against the adult of *A. persicus*.

Materials and Methods: Air-dried plant material were distilled using Clevenger apparatus. Chemical composition of essential oils were identified with a GC/MS system. The ticks were collected in Markazi province in the central of Iran and exposed to three-fold serial dilution of oils (5-80 $\mu\text{l}/\text{cm}^3$) using a dipping method in vitro. The adult ticks were immersed in different plant dilution (five per dilution) for 1 min then each replicate was incubated in separate petri dishes at 26°C and 80% relative humidity.

Results: Macroscopic observation indicated that in effective concentration of herbs essential oils, patchy hemorrhagic swelling appeared on the skin of treated ticks. The value of LC_{50} on adult was 7.03695 $\mu\text{l}/\text{cm}^3$ for *Eucalyptus* and 9.76496 $\mu\text{l}/\text{cm}^3$ for *Mints*. Also with increasing oil concentration, mortality rate of adult was increased.

Discussion: The results showed that both plants, Particularity *E. caesia* Benth can be considered as potential candidates for biocontrol of *A. persicus* in the field.

Keywords: *Mentha piperita*, *Eucalyptus caesia* Benth, *Argas persicus*

First molecular and morphological evidences of *Acanthamoeba* T3, T4 and T5 genotypes in hydraulic system of hemodialysis units in Iran

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Abstract

Background: *Acanthamoeba* is a genus of the free-living amoeba that widespread in the environment and causative agent of opportunistic infections in human. This study aimed to investigate the existence and genotyping of *Acanthamoeba* species in hemodialysis units in Iran.

Methods: In the present study, forty water samples of hydraulic systems and twenty dust samples were collected from two hemodialysis units in Mazandaran Province, northern Iran. The samples were cultivated on non-nutrient agar and genotyping was performed by targeting the 18S rRNA gene.

Results: Both morphology and molecular analysis showed that 17.5% (7/40) of water samples and 50% (10/20) of dust samples were positive for *Acanthamoeba* spp. The sequencing analysis of these isolates was found to be T3, T4 and T5 genotypes.

Discussion: To the best of our knowledge, this is the first investigation to identify of *Acanthamoeba* species in hydraulic system of hemodialysis units in Iran. High contamination of hemodialysis units with virulent T4 genotype of *Acanthamoeba* may pose a risk for biofilm formation. Our results support urgent need to improve filtration methods in dialysis units and monitoring hemodialysis patients for *Acanthamoeba* infections.

Keywords: *Acanthamoeba*, genotypes, hemodialysis units, Iran

Global prevalence of *Leishmania* RNA virus in *Leishmania* parasites: a systematic review with meta-analysis

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Leishmaniasis is one of the most neglected tropical diseases caused by protozoan parasites belonging to the genus *Leishmania*. There are several evidences regarding prevalence of *Leishmania* RNA virus (LRV) causing Old World (OWL) and New World Leishmaniasis (NWL), however a combined evidence-based knowledge on this topic is not still available. The purpose of this systematic review and meta-analysis was to address the global prevalence of LRV in the available literatures.

The data were systematically collected from the English electronic databases up to May 2018. Then, the studies were screened based on the inclusion and exclusion criteria. The random effect model was used by forest plot with 95% confidence interval (CI).

Overall, 877 samples from 17 articles were included for this study. Given species of *Leishmania*, the highest prevalence of LRV was belonged to *L. guyanensis* and *L. braziliensis*. Additionally, the virus was detected also in *L. amazonensis*, *L. panamanensis*, *L. lainsoni*, *L. aethiopica*, *L. major* and *L. infantum*. By random effect model, the global prevalence of LRV was estimated to be 26.2% (95% CI: 14.4–40.1).

The high prevalence of LRV among causative agents of NWL isolated from the metastatic clinical forms suggests potential association of LRV with metastatic clinical forms in New World endemic regions. However the LRV was found in OWL and non- metastatic lesions, subsequently a comprehensive investigation about experimental and clinical aspects of LRV is needed to fully appraise the role of these viruses to pathogenicity of Leishmaniasis.

Keywords: Leishmaniasis, *Leishmania* RNA Viruses, Systematic Review, Meta-Analysis

Prevalence and Rate of Parasitemia of *Haemoproteus columbae* in *Columba domestica* in Bandar Torkaman

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Haemoproteus columbae is the causative agent of pigeon Malaria occurs in pigeons widely in tropical and subtropical regions. The present investigation was concentrated on the prevalence of *H. columbae* and rate of parasitemia in pigeons in Bandar Turkaman, Iran. 100 pigeons sort in three groups. Blood smears fixed in absolute methanol and stained with giemsa. Data were analyzed with SPSS. The results of this study indicate that 89 (89%) pigeons were infected by *H. columbae*. Mean of parasitemia in infected females 49 (49%) were more than males 40 (40%). There was no significant difference in infection status between the two sexes ($P > 0.05$). Mean of parasitemia in infected pigeons were 3.39%. The highest percentage of infection with this parasite in Guilan was (78.33%). The present study shows the level of contamination more than previous reports. The prevalence of *H. columbae* in pigeons to be 17.47% and 20.8% and 62% in Golestan and Ilam and Isfahan provinces of Iran, respectively. The prevalence of *H. columbae* infection in pigeons has been reported to be 75% in Botswana, 70.4% in Turkey. In this study, infection rate in females was more than males and this may be due to longer exposure of females to vectors while sitting on the nest.

Therefore, birds may be considered as a biological transporter of *Haemoproteus* spp. that could transport infection to other birds. Finally, it is necessary to controlling the spread of *H. columbae* through identification and treatment of infected domestic pigeons.

Keywords: Avian Malaria, *Haemoproteus columbae*, Bandar Torkaman, Golestan, Iran

Higher levels of arsenic and nickel contents in the larval nematode parasites compared to tissues of *Psettodes erumei* fish in the Persian Gulf

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Introduction: The persistent and accumulative nature of heavy metal pollution in the aquatic ecosystem has attracted serious concerns in recent years. There has also been increasing interest in the role of parasites as bioindicators of heavy metals in aquatic habitats and especially in the interrelationship between parasitism and pollution. Limited information is available regarding heavy metal in the parasite and host fish in Iran. This study is aimed at evaluating the arsenic (As) and nickel (Ni) concentration and accumulation in fish and their parasites.

Material and method: Thirteen fishes (*Psettodes erumei*) were randomly collected from the Persian Gulf. Fish muscle and endoparasite samples were prepared and analyzed for levels of As and Ni. These metals concentrations were measured from the digested samples and then aspirated into the flame of the Atomic Absorption Spectrophotometer (AAS).

Result: The presented results showed that the larval nematodes are able to accumulate heavy metals in their tissues. As and Ni were detected at significantly higher levels in the parasites compared to host tissues ($P_{\text{value}} < 0.05$).

Conclusion: This study shows that larval nematode parasites can accumulate these metals from its host, reducing the concentration of these pollutants in the tissues. They may also have a beneficial effect on the health of their hosts by acting as heavy metal filters. The higher heavy metal levels in endoparasites indicate the potential risks of metal accumulation from fish consumption and suggest parasite suitability as bioindicators of heavy-metal pollution in aquatic ecosystems.

Keywords: *Psettodes erumei*, larval nematode, heavy metal, Persian Gulf

Identification and Characterization of Intracellular Proteases in Crude Extract of *Acanthamoeba* Trophozoites Isolated from Clinical and Environmental Sources

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Abstract

Acanthamoeba is a common free living amoeba that distribute widely around human habitats. Several species of *Acanthamoeba* are capable to cause serious as well as fatal infections in human beings. The aim of present research is to identify intracellular proteases of *Acanthamoeba* T4 isolates from different sources as well as to correlate pathogenicity with protease banding pattern.

In total, 19 isolates that had been isolated from environmental sources (8 isolate) and keratitis cases (11 isolates) were axenically cultivated, then crude extract (CE) was prepared from trophozoites. Subsequently, proteases identification was done by zymography technique. Further characterization of the proteases was carried out using phenylmethylsulfonyl fluoride (PMSF) as serine protease inhibitor. Zymography analysis revealed the presence of 3-5 distinct proteolytic bands in CE samples. Also, protease activity of all isolates was entirely inhibited by PMSF which represent their serine protease nature.

In conclusion, the complexity of protease banding patterns was not allowed to establish an indicator protease or banding pattern for differentiation of *Acanthamoeba* isolates from different sources or different pathogenicity potential.

Keywords: *Acanthamoeba*, intracellular, serine proteases, crude, extract

Isolation and Molecular Characterization of *Acanthamoeba* from Soil and Hospital Environment, North West of Iran

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Acanthamoeba spp. are omnipresent, free-living amoebae (FLA) with worldwide distribution. The amoeba has been frequently isolated from various natural and man-made habitats.

In this cross sectional study, 8 soil samples were taken from children's playground of recreational parks and university campus. Additionally, a total of 16 dust samples were prepared from two ophthalmology centers and a general hospital. The samples were collected from the surface of slit lamps, patient rooms, nursing station, patient waiting rooms, and a burn intensive care unit. The soil and dust samples were cultured and regularly monitored for the presence of *Acanthamoeba* cyst or trophozoite. For molecular characterization, PCR and sequencing were performed based on *Acanthamoeba* specific amplicon (ASA.S1) of Rns gene.

Cysts and trophozoites of the amoeba were observed in the cultures of 6 (75%) soil samples and 2 (12.5%) dust samples. In PCR examination, 5 soil samples and 2 dust samples showed expected products. Sequencing of PCR products of soil isolates revealed that 4 isolates belonged to genotype T4 (80%), and one isolate to genotype T3 (20%). Also, both of the dust isolates belonged to genotype T4 (100%).

The study revealed a high degree of contamination in both soil and dust samples in the city of Tabriz, north western region of Iran. It should be taken into more consideration that the detection of potentially pathogenic genotypes of *Acanthamoeba*, can be a risk factor for hospitalized patients and susceptible people exposed to soil.

Keywords: *Acanthamoeba*, soil, dust, Iran

Misdiagnosis of *Acanthamoeba* keratitis as herpes simplex virus keratitis

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Background: *Acanthamoeba* keratitis (AK) is a parasitic infection is seen most often in contact lens wearers. AK may cause permanent visual impairment or blindness, due to damage to the cornea or through damage to other structures important to vision. It is often characterized by pain out of proportion to findings and the late clinical appearance of a stromal ring-shaped infiltrate. Diagnosis and treatment of Ak are also difficult.

Material and Methods: In March 2019, a 31-year-old woman with a symptom of sensitivity to light, excessive tearing, blurred vision with eye redness and pain sensations in the eye was referred to Farabi Hospital in Tehran, Iran. The patient had a history of soft contact lens use and combination of steroids and antivirals for the treatment of Herpes simplex keratitis. After the diagnosis of Ak using in vivo confocal microscopy, corneal scraping was performed. The presence and genotype of the amoeba have been confirmed by culture and PCR Methods.

Results: After Ak is confirmed, the disease was controlled by chlorhexidine, Poly hexamethylene biguanide, and oral voriconazole drugs. Because of the initially misdiagnosed of the AK and used of steroid for treatment, the patient became Penetrating keratoplasty to prevent the progression of the Ak. The patient's cornea was completely cleared after a month.

Conclusion: To prescribe correct medications for the treatment and prevention of disease progression, the correct diagnosis of Ak is very essential. Use of culture and molecular methods also help to identify AK correctly beside confocal microscope, especially in the early stages of Ak.

Keywords: *Acanthamoeba* keratitis, herpes simplex, diagnosis

Molecular genotyping and Seroprevalence of Toxoplasmosis in mothers and their spontaneous aborted fetuses in Kohgiluyeh and Boyer-Ahmad Province, southern Iran

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Introduction: Toxoplasmosis is a zoonotic parasitic disease that affects millions of pregnant women and their children. Given the lack of routine screening of *Toxoplasma* infection in pregnant women in Iran this study aimed to evaluate the rate and features of *Toxoplasma gondii* infection in the spontaneously aborted human fetuses in Kohgiluyeh and Boyer-Ahmad Province, southern Iran.

Materials and Methods: This cross-sectional study was conducted on 100 spontaneously aborted fetuses' tissues and their mother blood samples. The mothers' sera were tested for anti-*Toxoplasma* antibodies while their buffy coat and the aborted fetuses' tissues were evaluated for detection of *T. gondii* DNA. Likewise, real time PCR assay was performed to quantify the parasite burdens in mothers' buffy coat and fetus tissues.

Result: Most of the mothers were 25-35 years of age and abortions occurred in the first trimester of their pregnancy in all pregnant women. ELISA, Real-Time PCR, and conventional PCR methods revealed *T. gondii* in 10%, 3%, and 2% of the studied pregnant women, respectively. Real-time PCR detected *T. gondii* DNA in the buffy coat of one seronegative case and 2 (out of 3) IgM seropositive cases. None of the samples from aborted fetuses were infected with *T. gondii* using PCR and Real Time PCR methods. Sequencing analysis showed that both positive samples belonged to type I of the *T. gondii* genotype.

Conclusion: Findings of this study demonstrated type I genotype of *T. gondii* in two mothers with spontaneous abortion, without fetus involvement. Evaluation of more aborted fetuses' samples from different geographical areas is needed to elucidate the association between *Toxoplasma* genotype and abortion.

Keywords: Abortion, *T. gondii*, Fetus, PCR/ Real Time PCR, ELISA, Sequencing, phylogenetic analysis

Fauna Survey of Argasidae and Ixodidae ticks parasites and their seasonal activities in Tehran province, Iran

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Objectives: Ticks are important hematophagous arthropods belonging to the class Arachnida. They transmit different groups of micro-organisms such as arboviruses, bacteria and parasites to animals and humans. A survey on tick species composition was carried out in Tehran Province Iran during year 2014-2015. The aim was to investigate faunistic of Argasidae and Ixodidae ticks and their seasonal population dynamics in Tehran province.

Matertials and Methods: In this survey, after data collection, geographical, ecological and density of animal units in Tehran province, samplings were carried out randomly in each zone and all seasons of the year by multi-stage method. Collected ticks were labeled and transferred to the Entomology laboratory, School of Public Health, Urmia University of Medical Sciences for species identification using different morphological characteristics via a stereomicroscope and systematic keys.

Results: The overall 574 ticks (38.5% male, 47.21% female and 14.21% Argasidae) were collected. Six Hard and soft tick genus were identified including: *Rhipicephalus*, *Hyalomma*, *Haemaphysalis*, *Boophilus*, *Argas* and *Ornithodoros*. *Rhipicephalus sanguineus* was the most abundant species while *Boophilus annulatus* was the least species. *Rhipicephalus sanguineus* species was the dominant species of ticks in both mountainous and plain zone of the study area. The seasonal activity of ticks in spring was more than other seasons and the least was observed in winter. The tick species diversity on sheep was the most whereas cow had the least tick species diversity.

Discussion and conclusion: These all indicate that the bionomic situation of the studied areas were favorable for the successive establishment of diverse ticks population. Finally, determining the fauna and geographical distribution of ticks in a given area is crucially important for monitoring control programs and prevention of tick-borne diseases.

Keywords: Argasidae, Ixodidae, Seasonal Activity, Ticks, Tehran, Iran

Determination of infectious rate of bilharziasis due to *Schistosoma haematobium* with emphasis to *Bulinus truncatu* snails in 2 former endemic foci of Dezful and Shuh, Khuzestan province SW Iran (2013-14)

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Introduction and Aims: This research has been done by cooperation Third Millennium Health Development Institute and Iran Ministry of Health to provide a document as a successful eradication bilharziasis in Khuzestan province of Iran in 2 former endemic foci of Dezful and Shoosh, Khuzestan province SW Iran from point of PCR.

Research and methods: Totally, 77 samples of *Bulinus* snails were collected from different regions of Dezful and Susa. In the initial study of a microscopic search method was used to find the cercaria. Then all infected samples were examined for the steps of PCR.

In *Schistosoma* microscopic examination snail cercaria were detected from *Shistosomatidae* family. In the PCR, primers were designed.

Then, 15 samples were positive after applying PCR phases. They have a band of between 450 bp and 400 bp.

To ensure the correctness of the reaction, the second pair of primers used to diagnose the genus of the *Schistosoma* was also used.

To detect the species of *Schistosoma haematobium* from other species in the region a special primer was also designed. Two PCRs were performed to ensure the correctness of the work. In all cases, 550 BP band did not detect *S. haematobium*.

Results: The results showed that the taken bands were 550BP that belonged to animalia species of *S. nazalis* and *A. bovis* special from buffalo.

Discussion: But it is concluded the control of bilharziasis by Iranian ministry of Health has been carrying out correctly, but the month checking should be reprogrammed.

Knowledge, attitude and practice of pastoralists about parasitic diseases and antiparasitic resistance: a study from Hamedan province

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Background and objectives: Role of pastoralists in development of antiparasitic resistance as an ongoing concern in the world, has not got adequate attention. This research aimed to assess knowledge, attitudes and practices of farmers in Hamedan province about parasitic diseases and antiparasitic resistance.

Methodology: Individual interviews with 150 pastoralists of different geographical areas of Hamedan were performed in June and July 2019 using a structured questionnaire. Descriptive analyses were performed using SPSS software.

Results: Most of the respondents were older than 40 years, had over 10 years experience, kept cattle and sheep and about one third were illiterate. Around half never heard about antiparasitic resistance but were interested to know about it. Most of pastoralists knew that debility, inappetence and cachexia were signs of parasitism but icterus and abortion were unknown for them. Approximately 70% said new-arriving livestock should be quarantined. More than half did not know zoonotic parasites exist. Almost all believed consult with veterinarian prior to using antiparasitics is necessary and educating farmers is the best way for control of parasitic diseases and prevention of antiparasitic resistance. More than half believed self-medication and use of antiparasitics without prescription by veterinarian may be related to development of antiparasitic resistance while remaining believed it is okay or did not have any opinion. Approximately 50% believed expensive drugs are better than cheap products. In practices area, almost 90% said they always or usually self-treat their livestock and over 60% treated livestock before definite diagnosis. Less than 10% always quarantined new-arriving livestock, 70% never or rarely used theileriosis vaccine. Almost one third sometimes or rarely performed ectoparasitic control by spray. Albendazole and ivermectin were the most used antiparasitics. Approximately 25% fed dogs and cats with infected organs of livestock.

Discussion and conclusion: For better control of parasitic diseases and prevention of antiparasitic resistance, knowledge, attitudes and practices of farmers need improvement. This can be achieved providing educational programmes by related organisations such as Ministry of Agriculture, Veterinary Organisations as well as public and social media. Also availability of drugs especially over-the counter anthelmintics to farmers should be under more regulatory supervision.

Keywords: Antiparasitic, Hamedan, KAP, Questionnaire, Resistance

Study of genetic diversity of *Cryptosporidium* spp isolates in Small Ruminants by application Sequencing of genetic markers 18S rRNA and GP60 in Isfahanprovice

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Introducion: *Cryptosporidium* infection are the important intestinal protozoan with a different host range, having the capability to infect humans and different animals.among the different animals sheep and goats are considered to be one of major animal reservoir hosts of *Cryptosporidium. cryptosporidium* in animals are of major concern due to the huge number of domestic animals and their economic importance.

Methods: In this survey, faecal samples from 75 sheep, 75 goats were investigated. Samples were stained with the modified Ziehl–Neelsen method and tested for *Cryptosporidium* infection using PCR with specific primers. PCR-RFLP of the 18srRNA gene was used for species identification and Nested PCR and sequencing of the 60 kDa glycoprotein (GP60) gene was used for subtyping on *Cryptosporidium*-positive stool samples.

Results: In sheep and goats seven (%4.6) of the specimens were positive for *Cryptosporidium parvum*. the prevalence of *Cryptosporidium parvum* in diarrheic animals were significantly higher than other groups. sequence analysis of the GP60 locus identifiedtwo different subtypes were identified: IIaA15G1R1 (4), IIaA15G2R1 (3)

Conclusion:. High prevalence and identical genotypes of *C. parvum* in animals indicate zoonotic transmission due to contact with animals, involving IIaA15G1R1 and IIaA15G2R1. Considering the zoonotic significance of *C. parvum*IIa subtypeis most common in animals and prevention and continuous monitoring of *Cryptosporidium* are required.

Keywords: *Cryptosporidium*, genotypes, *gp60* subtypes,PCR-RFLP

In silico and in Vitro Antileishmanial Evaluation of a copper (II) complex

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Introduction: Cutaneous Leishmaniasis (CL) is a challenging health issue in many countries with annual incidence of upto 1.2 million cases worldwide. Metal based drugs, led by meglumine antimonate (Glucantime), have a promising history in the treatment of Leishmaniasis and still good candidates for drug discovery. Likewise, copper complexes are important biologically active compounds with a wide range of potential applications, which deserve to be tested for antileishmanial properties.

Methods: Anti Leishmanial activities of Cu (II) coordinated by two dimethyl-bipyridine ligands (MEBPY) were tested on *L. major* promastigotes inoculated in RPMI medium supplemented with 10% fetal calf serum at 10⁶ parasites /mL. The promastigocidal effects were evaluated using MTT assay over 24, 48 and 72 hr. IC₅₀ value was calculated as the concentration capable of inhibiting 50% of parasite growth. Molecular docking simulation analyses were also carried out against a protozoan metacaspase as key macromolecule putatively involved in apoptosis to examine whether the compound promotes apoptosis.

Results: Significant ($P < 0.05$) inhibition of the growth of promastigote of *L. major* by the applied complex in a dose-dependent response manner was observed. Docking studies indicated a potential tendency of the tested drug candidate towards the opted metacaspase.

Discussion and conclusion: The metal cored complex studied in this project showed anti Leishmanial properties and proved itself a candidate for further studies. It's in silico interaction with the chosen metacaspase could be suggestive of its apoptotic action. These findings provide the scientific evidence that this metal-based complex drug could be subjected to further studies in a hope for treatment of CL.

Keywords: Leishmaniasis, MTT, Docking, Complex, Copper

In vitro and computational anti Leishmanial studies on an organometallic Cu (II) complex

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Introduction: Leishmaniasis caused by members of protozoan *Leishmania* genus results in a serious illness, principally in the tropical and subtropical areas. Metal cored complexes, like meglumine antimonate (Glucantime) with proven anti Leishmanial activity, keep being studied in vitro, in vivo and in silico to probe their potency as antileishmanial drug candidates. Molecular docking is an in silico algorithmic molecular modeling methodology that is also employed to predict the preferred orientation of usually a small organic molecule with biological macromolecules.

Methods: A copper (II) complex coordinated by dimethoxy-bipyridine (MOBPY) ligands was tested. Promastigotes of *L. major* were grown at 25 °C in RPMI supplemented with 10% heat-inactivated fetal bovine serum. To evaluate the anti-promastigote effects, colorimetric cell viability MTT assay was utilized in comparison with Amphotericin B. Viable promastigotes were then measured after 24, 48 and 72 h of incubation at 25 °C. Ligand-Host molecular docking analysis was carried out to study the effects of this complex on a protozoan apoptotic protein, metacaspase.

Results: The complex showed significant ($P < 0.005$) cidal effects against promastigotes as compared to Amphotericin B. Molecular docking revealed potential tendency between the target macromolecule and the complex used.

Discussion and conclusion: The in vitro and in silico findings of the present study demonstrated that the tested compound can be introduced as a new Leishmanicidal agent provided further studies approve its safety and efficacy.

Keywords: Leishmaniasis, MTT, Docking, Complex, Copper

Paleoparasitology of Chagas disease-A Review

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Chagas zoonosis disease is of the most important neglected disease with wide spread in South America The causative agent is the kinetoplastid *T. cruzi*.

It has been proposed that the *cruzi* clade originated on the super-continent formed by South America, Australia and Antarctica. The first American settlement was in coastal region of the Atacama Desert in where they could be contaminated with *Triatoma* bug. There is an idea that first contamination of human is at the entrance time to America in pre-Columbian era. The next spread of human was toward south and north of America about 12000 years ago.

It can be concluded that infected human could have an important role in spreading of Chagas disease.

Molecular study of Chagas disease can bring out some flourishing idea about the genetic evolution and its impact on the clinical features during adaptation to the hosts.

Keywords: paleoparasitology, Chagas disease, *Trypanosome cruzi*

New Species of *Kalicephalus* (Diaphanocephalidae), from viper Snake, *Macrovipera lebetina* (Family: viperidae), on Khuzestan Province, Iran

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Introduction: Some species of Strongylid nematodes of wild animals can be threaten reptiles' health as well as the human. Infection with *Kalicephalus* spp., the common gastrointestinal hookworms of reptiles caused mild enteritis, respiratory disorders and even death on its host. Although nearly 50 species of *Kalicephalus* have been identified throughout the world, there is very limited reporters of infection of this parasite in Iran.

Material and methods: In the present study two died Viper snakes due to various incidents were referred to parasitology laboratory. After examination and necropsy, all visceral organs of body were dissected. A number of red nematodes with a white head (>1.5 cm long) were attached to the mucosa of stomach. Specimens for light microscopy cleared and mounted in lactophenol. Several specimens were fixed for molecular study and one specimen was prepared and referred to Scanning Electronic Microscope (SEM). Extraction of helminthes DNA and amplification of the internal transcribed spacers (ITS), were carried out. Then sequences were analyzed by phylogenetic tree.

Results: The amplicons of approximately 850 bp in length were seen in all samples. Based on morphological findings, *Kalicephalus viperae* sub sp. *Viperae* was identified. Sequencing and phylogenetic (Maximum likelihood) findings showed that this species belonged to separate clade. *K. viperae* was sister to *Ancylostoma* spp. and they were far apart from the *Cyathostominae* sp. ex monkey and *Oesophagostomum* species.

Conclusion: This is the first report of occurrence of *Kalicephalus* species in Viper snakes (*Macrovipera lebetina*) in Iran.

Keywords: *Kalicephalus viperae*, Snake, PCR

Molecular study on parasite infected snails of Bamdezh pond, in northwest of Ahvaz, southwest of Iran

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Introduction: Many species of infective trematodes pass some developing stages of life cycle in snails as intermediate hosts. Some intestinal trematodes are medically important parasite infecting humans and livestock. About 100 species of snails have been reported to act as intermediate hosts for the trematodes. A commonly practical method to detect the larval trematode infections in snails by exposing the snails to light or dissection based on morphological characters, though it is often time consuming and inaccurate, also finding the rate of infected snails with shedding cercariae method is very low. Specific identification of cercariae from field snails is essential for epidemiological studies. The molecular approach has helped to detect of cercarial stages in infected snails.

Material and methods: In present study a number of fresh water snails were collected from Bamdezh pond, in northwest of Ahvaz, southwest of Iran. After morphological identification of cercaria by crushing the snails under light stereomicroscope, two types of cercariae were collected and preserved in 70% alcohol for DNA extraction and PCR amplification of the 5.8s, ITS2 and 28s rDNA. Then sequences were analyzed by phylogenetic tree.

Results: Morphological results were detected only pleurolophocercous cercariae and one unknown species cercariae. Sequencing and phylogenetic findings showed that pleurolophocercous cercariae was sister with a clade where Haplorchistaichui was located and other species of cercariae was belonged to Dicrocoelidae Family.

Conclusion: So prevention of food borne parasitic disease is closely related to control of intermediate hosts.

Keywords: Haplorchistaichui, Dicrocoelidae, Snail

Molecular study on strongyle nematodes of a working donkey (*Equus asinus*) in Iran: A case report

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's health has led to high prevalence of gastrointestinal parasitic diseases. More than 90% of population of donkeys was infected with a wide range of strongyles worms in Iran. Strongylosis, in spite of threatening the animal's health and working performance, causing the serious economic losses. Differentiation of the strongyles very time consuming and requires experienced personnel, so the use of the genomic DNA technology as accurate tool for identification of various species of strongyle worms is necessary.

Material and methods: In the present study, a dead donkey was referred to veterinary hospital of university. After necropsy and dissection the visceral organs, a population of red and with nematodes with multiple nodules were seen in large intestine. Specimens for light microscopy examination mounted in lactophenol for wet mounting. Several ethanol specimens stored for molecular investigations. Extraction of helminthes DNA and amplification of the 26S rDNA and intergenic spacer and 18S rDNA regions were carried out.

Results: Based on morphological criteria given, the identified species include: *Cylicocyclus radiatus*, *Triodontophorus serratus* and *Cylicocyclus brevicapsulatus*. The amplicons of approximately 700 bp and 1400 bp sizes. Sequencing and blasting on amplified regions showed that the cyathostomin species were closely located with *cylicocyclus* spp. and the species of *Triodontophorus serratus* were identity with *Parapoteriostomum* spp. (Strongylinae).

Conclusion: The previous studies identified some species of strongyle nematodes in Iranian donkeys by morphological characteristics. This is the first molecular report of strongyle nematodes in donkeys in Iran.

Key word: Strongyle nematodes, PCR, Molecular, Donkey

The effect of a constant (0.3T) and frequency(1 , 2mT and 50,100 Hz) electromagnetic field on protoscoleces of hydatid cyst

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Introduction: The aim of this study was to investigate The effect of a constant electromagnetic field 0.3 Tesla's and alternating 1 and 2mT frequency of 50 and 100 Hz on protoscolecesof hydatid cyst.

Materials and Methods: Five infected livers with hydatid cyst were collected from slaughterhouses. Viability of protoscoleces was assessed by 0.1% eosin staining. Samples for 2 and 4 hours were exposure to EMF 50and 100 Hz, with an intensity of 0.3 Tesla and 1 and 2 MT. For each of the conditions mentioned above, there was also a control group with no exposure. protoscolosomal percent of electromagnetic field in each group was determined. Data were analyzed by t-test in SPSS18.

Results: The results of this study showed that The highest mortality rate is related to the alternative magnetic field of 2milli Tesla with a frequency of 100 Hz, and it was at the fourth hour of exposure, and The lowest percentage of fecundity is related to the alternating current of 1milli Tesla with a frequency of 50 Hz and it was at the fourth hour of exposure. Also, the results showed that intermittent magnetic field has a higher friction effect than a constant field, and this effect also increases with increasing frequency.

Conclusion: Magnetic fields with increased exposure time have a greater effect on the mortality rate of hydatid cyst protoscoleces. Also, according to the results of this study, the use of magnetic fields on the hydatid cyst protoscoleces has a deleterious effect.

Study of therapeutic effect of stem cells and nanoparticles of gold and selenium on Leishmaniosis and Toxoplasmosis infections

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In treatment of some parasitic diseases such as Leishmaniosis and toxoplasmosis, we face problems like drug resistance and high costs. On the other hand, the economic conditions of people with parasitic infections are not appropriate due to their income level. Therefore, experts are looking for new therapies with high efficiencies and less complications and costs. Stem cells have been tested for inflammation from the infection, inhibition of tissue immunity and antimicrobial properties in the treatment of some parasitic diseases. Nanoparticles also have high potential for treating microbial infections with high efficacy and targeting in the transmission of therapeutic agents. For example, a study was conducted to treat the effects of gold nanoparticles on rats infected with Leishmanial major, and the result was a reduction in ulcerative amastigotes. Articles related to key words such as parasitic infections, a new therapeutic approach, nanoparticles and stem cells, which were published in 2010 to 2016, have been extracted and used from local and foreign sites. The use of mesenchymal stem cell increases the level of secretion IL-10 and nitric oxide - which results in suppressing infection and increasing the capacity of the host immune system. Gold nanoparticles have had positive results in treatment and have reduced mortality in mouse-infected leishmaniosis. Toxoplasmosis under the influence of gold nanoparticles initially had a moderate effect and then a better result after effective laser treatment. Selenium helps prevent Leishmaniosis. The therapeutic potential of nanoparticles and stem cells is further confirmed in the study context and more research is needed for formal use.

Keywords: Toxoplasmosis, Stem Cell, Nano, Gold, Leishmaniosis

Study of Protoscolicidal activity of honey

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Introduction: Hydatid disease is still an important endemic problem in many other parts of the world. Up to date, many protoscolicidal agents have been used for inactivation of the cyst content, but there is no ideal agent that is both effective and safe. The aim of the present study was Protoscolicidal activity of honey

Materials and Methods: Four kinds of traditional honey as Thymus, Astragalus, forty plants compound and Citrus were selected. Any kind of honey were entered in experiment as without dilution and diluted by DW as 50, 25 and 12.5%. Exposure was enrolled in three exposure times of 5, 10 and 15 minutes with survival examination by eosin solution. Toxicity of all types was tested by intraperitoneal injection of 1% honey into 4 wistar rats for each type.

Results: Thymus and Astragalus honey at concentrations of 50 and 100% were totally effective against protoscolices at all exposure times. 25 and 12.5% of these types in exposure times of 5 and 15min had partially effect. Minimum protoscolices effect was belonged to Citrus honey. Polyphenols percent and Hydroxymethyl Furfural (HMF) content of Thyme and Astragalus types were significantly higher than two others. All types have not any toxicity effect on Wistar rats.

Conclusion: Chemical compositions of honey types were significantly related to their protoscolices activity. Thymus honey was shown to be effective against protoscoleces of hydatid cyst at all tested concentrations and exposure times and therefore was recommended as anti- protoscolices agent.

Keywords: Honey; protoscolicidal agent; Hydatid cyst

Prevalence of *Balantidium coli* of cattle population in Ilam Province

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Introduction: Protozoan parasitism covers a broad spectrum of diseases. Balantidiosis is a zoonotic disease and is acquired by humans via the fecal-oral route from the normal host, the pig, where it is asymptomatic. Water is the vehicle for most cases of balantidiosis. Human-to-human transmission may also occur. Transmission is direct, from a contaminated water or food supply to humans. However, several reports had been confirmed that, *B. coli* can emerge as a significant pathogen that is able to cause disease in uncommon mammalian hosts such as cattle. Based on the above findings and considering that some cases of this disease with symptoms of diarrhea, enlargement of abdominal cavity and anorexia were observed in Ilam's cattle, it was decided to carry out an epidemiological study on the disease.

Materials and methods: Because of their large size and spiraling motility, Balantidia can readily be recognized in wet mount slide preparations, even at a low magnification ($\times 100$). This is the case with freshly collected diarrheic stool samples, which are likely to contain actively swimming trophic ciliates, as well as bronchoalveolar wash fluid. Collection of stool samples was collected from 145 cows in different geographical regions of Ilam province. Samples were sent to the laboratory and the results were evaluated by statistical tests.

Results: The results of direct fecal examination showed that 17 of the livestock (11%) were infected with parasites. In the next step, the cattle were treated with a combination of oral sulfadimidine for 5 days and human metronidazole syringe, so that the result of the re-test was negative. Given the importance of this disease in terms of public health and its zoonosis, and because of the risk of contamination of food and water sources by domestic animals such as cattle, preventive measures are very important.

Keywords: *Balantidium coli*, cattle, zoonotic disease

Investigating the Effect of Nanoparticles of Ghalghaf Extract and Silver Oxide and its Combination on Hydatid Cyst Protoscolexes.

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Introduction and target: Cystic Echinococcosis is a parasitic disease that causes cysts in the human body and animals. Curing is mainly surgical. During surgery, there is a possibility of cyst fluid leakage into adjacent tissues and as a consequence of recurrence of the disease. In order to reduce the rate of recurrence, the use of Scolicidalin surgical procedures is essential. The aim of this study was to investigate the effect of methanolic and acetonic ghalghaf extract and silver oxide nano particle's concentrations and its composition on the number of protoscolexes in vitro.

Materials and methods: Protoscolexes were obtained from the liver from the slaughterhouse. Percentage of viable protoscolexes was evaluated 0.1 by Eosin color. Impacted Scolicidal was evaluated at three dilutions 0.1 0.01 0.001, at 1, 5, 10 and 20 minutes. Normal saline was used as a negative control and hypertonic salts as positive control.

Results: The highest percentage of germination was in the silver oxide nanoparticles with a concentration of 0.1 and at 20 minutes. Nanoparticulate integration of silver oxide and methanolic extract at a concentration of 0.1 and at 20 minutes was observed. So that the destructive effect of these two were significant and the time was not affected by the fatalities of these two groups.

Discussion and conclusion: The findings show that nanoparticulate of ghalghaf extract in combination with silver oxide, especially methanolic extract, can be used as a suitable protoscolicidal agent during hydatid cyst surgery using a pair of surgical techniques.

Keywords: *Echinococcus*, ghalghaf, hydatid cyst

Purification of Hydatid Cyst Fluid antigens and investigation of their effect on growth of Breast Cancer cell line in BALB/c Mice

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Background: Helminthic parasites possess some immuno-regulatory mechanisms which can change the immunological pathways. According to the hygiene hypothesis, in populations where the helminthic infections are non-endemic, the modulation process of immunological responses is disrupted and improper responses such as autoimmune diseases and malignancies occur. Parasites such as *Trypanosoma cruzi*, *Plasmodium yoelii*, *Acanthamoeba castellanii*, *Toxoplasma gondii*, *Toxocara canis*, as well as the *Trichinella spiralis* had antitumor activity against cancer. Recent surveys highlighted that helminths are immunomodulators and they possess some molecules that can interact with host immune cells. These molecules can be used to treat or prevent some inflammatory diseases. Hydatid cyst is the larvae of *Echinococcus granulosus*, which is a causative agent of hydatidosis. The hydatid cyst fluid (HCF) antigens have the ability to stimulate the humoral and cellular immune responses in humans and livestock.

Material and Methods: The HCF was collected from Rasht industrial slaughterhouse. After centrifugation, lipids were removed using ether. The purification of B and arc 5 antigens has been done using Sephadex G-200 for gel filtration. The Bradford protein assay was used to determine the protein content of each fraction. To evaluate the characterization of purified antigens, the SDS-PAGE method was performed.

Results: Three peaks of antigen fractions were obtained from gel filtration. The 27 kD and 50 kD molecular weights were considered as candidates in SDS-PAGE method.

Discussion: There are antigenic similarities between some types of cancers and the hydatid cyst. These antigens expressed in the early stages of various malignancies, including cancers. Most studies have been conducted on the anti-cancer effect of the HCF crude antigen, and so far no research has been done on the effect of specific antigens of HCF on various types of cancers, considering the importance of it in this investigation.

Conclusion: The antitumor activity of isolated antigen fractions in BALB/c mice model of breast cancer will be evaluated to find the best candidates. Also, the most important angiogenesis inhibitor and stimulant cytokines can be determined.

Keywords: Hydatid cyst fluid, Breast cancer, BALB/c mice

Semi-Nested Multiplex Polymerase Chain Reaction For Detection Of Human Malaria Parasites and Evidence Of *Plasmodium vivax* And *Plasmodium falciparum* Infection in South East Of Iran

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Introduction: Microscopy and rapid diagnostic tests (RDTs) are common tools for diagnosing Malaria, but are deficient in detecting low *Plasmodium parasitaemia*. In this study semi-nested, multiplex PCR based on the amplification of the sequences of the 18S small subunit ribosomal RNA (ssrRNA) gene was tested in a field trial in South east of Iran.

Methods: Blood samples (n = 300) were collected in South east of Iran from febrile patients without *Plasmodium* detectable by microscopy or RDTs. 18S rRNA gene sequences were amplified from extracted DNA by Semi-nested multiplex PCR to detect and differentiate *Plasmodium* parasites.

Results: A total of 300 samples tested by microscopy, RDT, and Semi-nested multiplex PCR were analysed. Among the negative samples detected by microscopy, 58 (19.3%) were false negatives. On the other hand, the negative samples detected by RDT, 29 (9.6%) were false negatives based on Semi-nested multiplex PCR.

Conclusions: Semi-nested multiplex PCR enhances low-parasitaemia Malaria diagnosis and can potentially surmount the deficiencies of microscopy and RDT-based results in determining low-parasitaemia *Plasmodium* infection rates for evaluating malaria elimination efforts. The findings highlight the need for improved differential diagnostics of febrile illness in remote Malaria endemic regions. The PCR method showed greater sensitivity and specificity than microscopic examination and confirmed the existence of a focus of *P. vivax* and *P. falciparum* South east of Iran infections in suspected by microscopic examination.

Keywords: *Plasmodium*, Malaria, Semi-nested Multiplex PCR

Epidemiology of Pulmonary hydatid cyst in Imam Reza Hospital of Kermanshah- Iran during 2013-2018

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Introduction and purpose: Hydatidosis is one of the most important parasitic disease. This zoonosis disease is endemic in some parts of the world, including Iran and is the cause of hospitalization of almost 1% of patients in surgical wards. The purpose of this study was to study the epidemiology of pulmonary hydatid cyst in patients admitted to Imam Reza Hospital of Kermanshah-Iran, during 2013-2018.

Materials and Methods: In this retrospective and descriptive study, the medical files of 123 patients with diagnosis of pulmonary hydatid cyst during 2013-2018 were studied. The study population were patients admitted to Imam Reza Hospital of Kermanshah-Iran. Data were analyzed using descriptive statistics.

Results: Among the 123 hospitalized patients, 56.9% were female, 74.8% married, 46.4% illiterate or elementary level, 52.8% unemployed or housewife, and 65.9% urban residents. The mean age of patients was 21.49±44.55 years. Four patients (3.3%) had liver and lung cyst hydatid simultaneously. The most common complaints were pulmonary distress (30.1%), epigastric pain (22%), cough with Hemoptysis (10.5%), and chest pain (8.9%), respectively. The mean duration of hospitalization was 6.17±9.87 days. The most common treatment was surgery or cyst aspiration (70.7%).

Conclusion: Most of the patients have low level of literacy and were female and housewife or unemployed. So it is suggested that educational programs be prepared for the community, especially low-literate women and housewives people.

Keywords: Epidemiology, Pulmonary hydatid cyst, Iran

A Review of the Situation of Current Babesiosis Sheep in Ilam city

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Introduction: Babesiosis is a important and dangerous parasitic disease in the tropical regions of the world, including Iran and It can bring a lot of economic losses to the animal husbandry industry. The carrier of that is hard tick from the ixodide family, which, from the bloodthirsty babesia getting in to the blood flow. Clinical signs are fever of 41 degrees and yellow mucus and hemoglobinuria that In the chronic form, due to anemia the animal going to loose weight and finally wasted. *Babesia* disease is known in different regions of Iran with variety name that every year in warm season especially in the late spring causing a lot of heavy casualties of sheeps.

Targets: Epidemiology status of *Babesia* in sheep in Ilam city

Research Methodology: In this study, 50 bloods samples were taken from the sheeps flocks of the Ilam city (dare shahr , soroor abad, jafar abad, gholam abad , taleghani) recived in a randomized manner. Then, from each blood sample, 2 spreads were prepared. Sampling was carried out in the seasons of activity tick. Blood spread was stained by Giemsa method and then morphological studies were carried out to find more objects.

Conclusion: Of the 50 blood samples, 23 cases were infected with Babesia parasites (46%).

Final conclusion: The results of this study showed that Babesian infection is present in Ilam sheep. Confront with carriers an important role in controlling the disease.

Keywords: Ilam, sheep, *Babesia*

***Leishmania major* can Be Traced in Fibroblasts: An *in vitro* Study**

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Back ground: Leishmaniasis is still a neglected tropical disease that can endanger more than 350 million people among 98 countries. It was shown that *Leishmania* can survive in fibroblasts as latent inactive forms. This study was conducted to evaluate the role of superparamagnetic iron oxide nanoparticles (SPIONs) in *in vitro* tracking of labeled *Leishmania major* in fibroblasts.

Methods: Dextran-coated SPIONs were used for labeling *L. major* in co-culture of fibroblasts with the parasite. To quantify and trace SPIONs-labeled *Leishmania*, Prussian blue staining was undertaken. Fibroblast characterization was undertaken by real time PCR. Transverse electron microscope (TEM) used for confirming the entry of the labeled *L. major* to the cytoplasm and the nucleus of the fibroblast.

Results: Fibroblasts were spindle-shaped and adherent to culture flasks. Promastigotes were thin elongated lance-like morphology with an anterior kinetoplast and an emergent free flagellum. Prussian blue staining revealed that internalized SPIONs were localized within cytoplasm and nucleus of the fibroblasts after 24 h culture. Prussian blue staining successfully showed the presence of iron (stained blue) in labeled *L. major* within the fibroblasts.

Conclusion: We can conclude that SPIONs are safe, inexpensive, easy to use, and accurate and a fast method to label *Leishmania* parasite in cells that the parasite can be latent such as fibroblasts.

Keywords: Iron oxide, Labeling, *Leishmania major*, Fibroblast, *In vitro*

Survey of *sarcocyst* in Livestock (cattle, sheep and goats) in Hamedan province in year 97

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Introduction: *Sarcosystis* is one of the most common parasites between humans and livestock that is create by various species of *sarcositis*. Some species of this parasite cause anorexia, weight loss, reduced milk production, muscle weakness, abortion, and sometimes death of host mediators such as cow and sheep. Pollution occurs in humans when it consumes meat and products from infected animals in raw or semi-raw form. This disease causes a lot of economic damage to the husbandry industry.

Targets: The aim of this study was to analyze pollution of *sarcosystis* in livestock in the slaughterhouse which provide protein that people need in hamedan.

Research Methodology: In this study conducted, in the autumn season of 1397, 111 specimens were obtained randomly from Hamedan's slaughterhouse. 16 cows, 65 sheep, 30 goats samples were collected. The age of cows was between 15 months and 2 years, and the age of sheep and goats was 9 months to 1.5 years. 94 heads of livestock, female sex (84.68%) and 17 heads were male (15.31%). In this study, the esophageal, aperture and muscle were examined for microscopic cysts by digestive method. The samples were digested with digestion method by normal saline after transfer to the lab and centrifuged 2000 round pair two minutes. Then from end of the test tube take a drop sediment checked on the lam for searching about bradyzoites.

Conclusion: The percentage of microscopic cysts in cows was 6 (37.5%) (25%) is muscle and 75% aperture pollution. The sheep are 50 cases (76.92%). 74.41% of the infection was muscle and 84.61% of the aperture and 77.77 of the pollution of the esophagus. And in goats there are 27 cases (90%). 75% pollution is muscle and 100% aperture pollution and 85.71% of the esophagus was observed. In the digestive method, the highest infected tissue was detected with aperture value (93.1%) and the lowest infection muscle tissue was detected 65.67%.

final conclusion: These results indicate high rates of pollution parasite in livestock that is need more searching on meats and focus about cooked them and improved situation of nutrition. keep livestock disconnect with main host can reduce pollution in livestock.

Key word: *Sarcocystis*, cattle, sheep, goat, Hamedan

Evaluation of molecular and serological diagnostic approaches for detection of *Toxoplasma gondii* in blood and different organs of sheep and goats in Ahvaz, South-West of Iran

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Abstract

Introduction: Toxoplasmosis is one of the most important zoonotic diseases with serious health problems especially in pregnant women and immuno-deficient patients in different countries worldwide. Human and different animals become infected through uptake of sporulated oocysts and/or eating undercooked/raw meat infected with *Toxoplasma gondii*. The present study aimed to evaluate *Toxoplasma* infection in blood and different organs of sheep and goats which slaughtered at Ahvaz abattoir.

Materials and Methods: A total of 150 samples of each organ including heart, liver and meat of sheep and goats were evaluated using amplification of a fragment of B1 gene by PCR. Moreover, 150 sera samples were tested using ELISA.

Results: A total of 26 (17.3%), 33(22%) and 48(32%) samples of liver, meat and heart tissues in sheep and a total of 24 (16%), 26(17.3%) and 36(24%) samples of mentioned tissues in goats have shown positive responses using PCR respectively. The ELISA evaluation using 150 sera samples has shown 20 (13.3%) positive cases.

Conclusions: Although, the infection risk in goat is less than sheep, goat also has specific importance regarding the habit of eating meat of both hosts in human beings. According to our results in compare with other findings, it is suggested that juice and sera samples be evaluated in the same study in future.

Keyword: *Toxoplasma gondii*, molecular study, serological study, sheep, goat

Systematic review of prevalence of cutaneous Leishmaniasis in Iran (1380-1396)

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Introduction: Cutaneous Leishmaniasis is a prevalent disease in Iran; So to get a better perspective on the prevalence of this disease, we reviewed a number of articles systematically.

Methods: To systematically review prevalence of Cutaneous Leishmaniasis in Iran, related articles to this disease were extracted from Pubmed, Google Scholar and SID databases. Finally 30 articles were selected and studied.

Results: In this study the total number of people suffering from Cutaneous Leishmaniasis was 62474. Nearly 58.47% Of them were men and 41.53% of them were women. 63.28% of this population were urban and 36.72% of them were rural. It was revealed that people with age between 0 to 20 were more exposed to infection and the minimum infection occurred in people aged Over 50 years. The prevalence of *Leishmania major* was more than *Leishmania tropica*. Regarding the number of ulcers, 46.38% Of patient had one lesion and 53.62% of patient had multiple lesion. Most of lesions were observed in hands, leg, face and other part of the body respectively. Housewives showed the highest degree of infection. These cases mainly reported in autumn.

Conclusion: Considering the high prevalence of Cutaneous Leishmaniasis in Iran, also psychological and social consequences for the affected people, appropriate health measures must be taken to control and treat the disease.

Keywords: Cutaneous Leishmaniasis, prevalence, Iran

Investigation the development and egg hatch assay of the egg of *Fasciola gigantica*

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Introduction: Fascioliasis is one of the most important food and water-borne parasitic zoonoses caused by the liver flukes of the genus *Fasciola*, it is about six million people in risk of *Fasciola* infection and Iran is one of the endemic areas in the world. This study was conducted to investigate the development and life history of the egg and the hatching condition of the miracidia of *Fasciola gigantica*.

Material and method: seven infective livers from sheep and 1 infective liver from calf were collected from an abattoir around Tehran, 251 *Fasciola* worms (*Fasciola hepatica* and *Fasciola gigantica* separated based on the morphological characterization) and about 10600 eggs were collected in sterile condition. The eggs divided in 10 petri dishes, about 1000 eggs in each petri dish. Five plates with normal saline and five plates with distilled water incubated in 23-24°C.

Results: This study developed an egg hatch assay with eggs recovered from adult worms. Embryonated eggs were observed a day after culture and about 22 days miracidium were observed. The process of the miracidia formation occurred in 60% of *Fasciola gigantica* eggs and in 40% of *Fasciola hepatica* eggs. There was 50% of *Fasciola gigantica* eggs and 33% of *Fasciola hepatica* were hatched in distilled water (pH 6.6 and 23-24°C).

Conclusion: By investigation the growth condition of *Fasciola* spp., it will be possible to take steps toward more advanced achievements for prevention and controlling the Fascioliasis.

Keywords: *Fasciola* spp., culture, Egg hatch assay, Temperature

Geographical Distribution and Molecular Identification of Causative Agents of Cutaneous Leishmaniasis in Different Areas of Fars Province, Southern Iran

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Background: Cutaneous Leishmaniasis (CL) is a vector-borne disease, caused by two main species of *Leishmania* genus as *Leishmania major* and *Leishmania tropica* in Iran. This study was aimed to identify the causative species of CL in cutaneous lesions of patients residing in different countries of Fars Province south of Iran focusing on the Shiraz city and county side using PCR technique.

Methods: This cross-sectional study was performed during December 2017 to April 2019 on 94 patients with CL referred to Diagnostic Lab of Leishmaniasis, Valfajr Health Center, Shiraz, Iran. After DNA extraction from all positive Giemsa-stained smears, the minicircle kDNA gene was amplified using LINR4 and LIN17 primers to differentiate *Leishmania* species.

Results: Of 94 positive direct smears of CL, 86 smears (91.5%) were also identified positive by PCR in all regions of Fars Province. Of 86 PCR positive samples, 49 samples (57%) were identified as *L. tropica* and 37 samples (43%) as *L. major*. All *L. tropica* specimens were from Shiraz city while all *L. major* specimens were from county side of Shiraz and other states of Fars Province. In Shiraz, the most prevalence of *L. tropica* was from old region of the city.

Conclusion: Both *L. tropica* and *L. major* were confirmed as causative agents of CL in Fars Province. *L. tropica* was detected only in Shiraz city whereas *L. major* was detected only in rural area of Shiraz and other states of Fars Province. This study show the importance of rodents as CL reservoir in the rural areas of the city.

Keywords: Cutaneous Leishmaniasis; *Leishmania tropica*; *Leishmania major*; Iran

A Survey of *Toxoplasma gondii* infection in aborted fetuses of sheep using ELISA method in different cities of North Khorasan Province

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Back ground: *Toxoplasma gondii* is a zoonotic obligate intracellular protozoan parasite that infects all warm-blooded animals as well as human worldwide. Human is infected with *Toxoplasma* parasites by eating half-cooked meat of livestock or oocyte.

Objective: The purpose of this study was to determine the presence of *Toxoplasma* in aborted fetuses of sheep using the serological method in North Khorasan province.

METHODS: In this study, from 2015 to 2017, 133 samples of the thoracic fluid in aborted fetuses of sheep from different cities of North Khorasan Province were collected and sent to the central laboratory of Bojnourd. For each sample, a questionnaire was prepared for gathering information such as age and city, and then, the antibody level was determined in each sample by ELISA method.

Results: In this study, the age of 133 aborted fetuses of sheep was more than 120 days. Also, of the 133 aborted fetuses of sheep, 14 samples (10.53%) were infected with *Toxoplasma* parasites. The highest and lowest rate of *Toxoplasma* infection was observed in 2016 and 2017, respectively. Also, the most infection was found in Shirvan and Faraj cities. Results of chi-square test showed that there was a significant difference between year and abortion in sheep due to infection with *Toxoplasma* parasite ($P < 0.05$). There was not significant difference between the frequency of this parasite infection and aborted fetuses in different areas ($P > 0.05$).

Conclusion: The results of this study indicate that *Toxoplasma gondii* infection is one of the causes of abortion of sheep in North Khorasan province, and since sheep is one of important sources of meat and dairy products in this area, Therefore, observance of public health tips and the complete cooking of meat and boiling milk is emphasized.

Keywords: *Toxoplasma*, ELISA, Abortion

Strongyloidiasis in immunocompromised hosts

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Introduction: *Strongyloides stercoralis* is an intestinal nematode endemic in the tropics and subtropics where hygiene standards and sanitary conditions are deficient. Infection in the immunocompetent host is usually associated with mild gastrointestinal symptoms.

This soil-transmitted helminth is the only parasite that secretes larvae in feces and alternates between free-living cycles (rhabditiform larvae) and parasitic cycles (filariform infective larvae). This review study focuses on describing *Strongyloides* infection in the immunocompromised host, including immune response against this infection.

Methodology: In this review study several databases including Pubmed, Elsevier- Scindirect, Google scholar, Scopus have been searched.

Result: Primary goal in organ transplantation is the prevention or effective treatment of infection, the most common life-threatening complication is long-term immunosuppressive therapy.

The challenges involved in achieving this goal are several: a broad range of potential sources of infection ranging from latent viruses to pathogens of both community and hospital origin infections.

Immunocompetent hosts typically are asymptomatic, despite chronic *Strongyloides* infection. In contrast, immunocompromised patients are at risk for hyper infection syndrome and disseminated disease, with a fatality rate of more than 50%.

Discussion and conclusion: There are small endemic foci of the disease in countries with a temperate climate. In immunocompetent individuals, this infection is often asymptomatic or has a mild symptomatology, characterized by high eosinophilia, pulmonary symptoms, dermatological manifestations, and intestinal symptoms. However, there are some risk factors, such as co-infection with HIV or human T-cell lympho-tropic virus (HTLV1).

Diagnosis is often delayed due to the nonspecific nature of presenting symptoms, and early diagnosis relies on a high index of clinical suspicion to prevent death.

However, newer diagnostic modalities, such as RT-PCR can be an effective tool for the early detection of transmission among solid organ transplant recipients.

A case of cutaneous Leishmaniasis caused by *Leishmania infantum*.

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Background and aim: Cutaneous Leishmaniasis (CL) is one of the most important parasitic diseases in tropical and subtropical areas of Iran. It caused by protozoan parasites of the genus *Leishmania* and characterized by ulcers, nodules or plaques on exposed regions of the body. Here we report a case of CL in a patient residing at Esfahan Province of Iran where this infection is prevalent.

Materials and method: A 29-years-old male presented at the pathobiology laboratory in Shahrekord University in January 2019 with three nodular lesions, two (19 and 26 mm in diameter, respectively) on the left arm and one (12 mm in diameter) under the left eye. Cutaneous Leishmaniasis was suspected and immediately the lesion and the adjacent skin were cleaned and sterilized with disinfectant. Smears were prepared from fluid materials of the skin lesions and stained with Giemsa. Skin lesion materials were cultured in special medium and Promastigote forms appeared after about 1 to 2 weeks. Finally, to identify the causative agent of the disease in above mentioned case polymerized chain reaction (PCR) was performed.

Results: Skin smears stained with Giemsa were viewed by using light microscope and showed Leishman bodies (amastigotes), so diagnosis was confirmed. Furthermore, PCR was positive for *Leishmania* and showed that the agent was *L.infantum*.

Conclusion: As Leishmaniasis is endemic in many areas of our country, we should be aware of the recent epidemiological changes about *Leishmania* infections. So, we present this case because the causative agent of cutaneous leishmaniasis was *L.infantum* in this case and the disease resolved without any treatments.

Keywords: Cutaneous Leishmaniasis, *Leishmania infantum*, PCR

The Prevalence of Leishmaniasis in Hormozgan Province during 2009-2018

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Introduction: Leishmaniasis is a common disease of humans and animals, which hosts a reservoir in wild animals and rodents. This parasite (*Leishmania*) produces complex diseases called Leishmaniasis. We studied the prevalence of cutaneous Leishmaniasis in Hormozgan province during 2009-2018.

Methods: This is a descriptive-analytic study conducted in Hormozgan province. To collect data in this study, the data of all patients registered in health centers of Hormozgan province during the years 2009 to 1397 were used. Then, the collected data were analyzed using SPSS-24 software and appropriate statistical tests.

Results: The total number of cases of Leishmaniasis was 1195, of which 664 (55.7%) were male and 529 (44.3%) were female. The highest incidence of cutaneous Leishmaniasis was related to Haji Abad city, 304 (3.8 per 100,000). Jask was 216 people (3.5 per 100,000) and Bastak was 223 (2.4 per 100,000). The incidence of disease during 2009-2018 showed that the highest number of cases was reported in 2009 (16.6%) and the least of them were in 2012 with 82 (6.9%).

And also showed that the highest incidence rate was observed in January and February (146). ($P < 0.001$). There was a significant relationship between the incidence of disease with the age of patients ($P < 0.001$) and sex ($P < 0.002$).

Conclusion: The results of this study showed that the age group under the age of ten and the students are more at risk of disease, therefore, they should perform appropriate health proceedings.

The Novel Methods of Control and Treatment of Leishmaniasis

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Leishmaniasis is a disease that ranges in severity from disfigurement skin lesions to serious and fatal systemic infection. Since there is no effective vaccine for prevention against Leishmaniasis, rapid diagnosis and appropriate chemotherapy, appear to be the only ways to control of the disease. The choice drugs have serious limitations including high cost, toxicity and many undesirable side effects. Undoubtedly, the most effective strategies to prevent the pathogenicity of harmful organisms is the development of effective vaccines against pathogens. In this way new generation vaccines can design and construct using as an effective control tool of Leishmaniasis. Macrophages, as a primary defense line, play a key role against infection produced by *Leishmania*. Targeting critical regulators of *Leishmania*-infected macrophages apoptosis may represent a useful strategy to drop or eliminate *Leishmania* infection. In recent years, research into antimicrobial peptides (AMPs) and animal venom toxins has intensified, focusing on the potential of these compounds to interfere with parasite physiology and/or vector biology. Hence many efforts have been made to increase effective new compounds for the treatment of Leishmaniasis that would be to develop novel drug delivery systems (DDS). In recent years, the explosive growth of nanotechnology has burst into challenging innovations in pharmacology, which is in the process of revolutionizing the delivery of biologically active compounds. Another method used to improve drug delivery mechanism is the use of pore-forming peptides. These peptides can generate a pathway for increased penetration of therapeutic agents across cellular membranes by creating pores in the cell wall. All the items in this summary will be discussed in detail.

Keywords: Novel Methods, Treatment, Leishmaniasis

Seasonal prevalence the Varrva mite in honey bee colonies in Maku Free Zone in 2018-2019

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Introduction: Theecto-parasitic mite *Varrva destructor* is a serious world-wide pest of the honey bee *Apismellifera* and has being linked with the death of millions of colonies, though its role is not completely destroyer of the colony. The aim of this study was to determine the seasonal infestation of bee colonies in Fars province to the *Varrva* mite is devastating the honey beeinmaku free zone.

Method: Samples of adult bees and pupae were collected from 80 apiaries in the spring, summer, autumn and winter during 2018-2019. Samples were taken from 20 apiaries in each season. For determining of infestation of adult bees, infected samples put inside the glass containing 70% ethanol. For determining of infestation of larval stages and pupae, in appropriate time from the side of the hive frame, a piece with dimensions of 5 × 5cm apart and with the ice was transported to the laboratory .

Result: Lowest rate of varroasis spread was in spring (22%) and it increases in the following seasons respectively and the highest rate was occurred in winter (46%).

Keywords: Honey bee, Prevalence, , Varrva, mite

Sonochemical preparation, characterization, and in vitro drug release of magnetic solid lipid nanoparticles co-loaded with Albendazole Sulfoxide

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Background & Objective: Albendazole sulfoxide (ABZSO) is an appropriate drug for use in anti-parasitic drug delivery system toward treatment of hydatid cyst. Since the efficient delivery, achievement of more drug loading, and controlled drug release of this drug need to application of the magnetic nanoparticles (MNPs) as carrier, and the best appropriate carrier was the magnetic solid lipid NPs (MSLNPs) core/shell structure multifunctional mesoporous nano-capsules, which was composed of inorganic (Fe₃O₄) NPs as the core and stearic acid as the shell, current study carried out to prepare magnetic solid lipid nanoparticles co-loaded with Albendazole Sulfoxide and determine characterization and in vitro drug release of this nanodrug.

Materials and Methods: Sonochemical preparation and synthesis of the of magnetic solid lipid nanoparticles co-loaded with Albendazole Sulfoxide (ABZSO-MSLNPs) accomplished by nanoemulsion using ultrasound. The MSLNPs were identified and recognized by energy dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM), vibrating sample magnetometer (VSM), Fourier-transform infrared spectroscopy (FTIR), and Dynamic light scattering (DLS) analyzer, which accordingly denote spherical particle morphology. Also, in vitro drug release of this nanodrug was investigated.

Results: The ABZSO-MSLNPs was homogeneously distributed with spherical shape and little agglomeration on SLNPs surface with average particle size less than 200 nm. Our in vitro release study demonstrated that 30-40 % of ABZSO-MSLNPs was released in 2 hours as rapid phase, and 85% of its gradually released within 36 h.

Conclusions: Our synthesis procedure is more rapid and environment friendly and possesses high efficiency for ABZSO delivery.

Keywords: Albendazole Sulfoxide; SLNs; Drug Release; Drug Delivery.

Clinical and molecular features of hydatid disease in Tehran from 2011 to 2019

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Background: Hydatid disease or Echinococcosis is a zoonotic disease caused by the larva of *Echinococcus* sp. This study aimed to evaluate the clinical and molecular features of hydatid cyst in Tehran.

Material and methods: In this cross-sectional study, the records hospital of all hydatidosis-affected patients admitted in hospitals of Tehran were reviewed between 2011 to 2019. Demographic characteristics, clinical findings, and laboratory data were collected. The descriptive statistical analysis was performed by SPSS software. For molecular characterization of isolates, specific primers were used for *cox1* gene amplification.

Results: Totally, 160 patients with the mean age of 45.00 ± 75 years were admitted with cystic Echinococcosis (CE) diagnosis. Moreover, the highest and the lowest prevalence of CE cases were in age ranges of 16–30 (1.9%) and 1–15 year (1.9%), respectively. Male/female ratio was 1.07 (51.9% vs. 48.1%). Nearly, fifty three percent of the patients ($n=86$), the affected organ was liver. The diameter of the cysts was variable from 1 to 25 cm. Most of the patients had a single hydatid cyst. Seven patients were infected more than 2 organs. The PAIR technique was applied for all cases. All patients underwent radical surgery. Based on molecular features, more than 80 percent of isolate belonged to G1 genotype.

Conclusion: hydatid disease is prevalent in Iranian population. Development of new diagnostic methods and therapeutic procedures is worthy. On the basis of molecular data, the domestic cycle is the main transmission route for Iranian patients.

Keywords: Cystic Echinococcosis, Prevalence, Clinical features, Iran

A case of multiple organs (disseminated) hydatid cyst in a general hospital

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Introduction and Objectives: Although liver and lungs are the main organs involved with the larval stage of *Echinococcus granulosus* (Hydatid cyst), the parasites rarely entered the general circulation and disseminated throughout the body due to trauma or medical manipulation (biopsy or aspiration) resulting rupture and transferring the protoscoleces or cyst/s content to other organs. This is especially considerable in immune- deficient/suppressed patients. In this regard, a case of disseminated hydatid cyst is presented.

Case report

A 29 years old woman with fever, chills, coughing, chest and back pain was referred to the emergency clinic at Baqiyatallah hospital. She was diagnosed with pulmonary inflammation and widespread infection with a history of rheumatoid arthritis (under treatment) for 3 years, eosinophilia (>14%) and multiple pulmonary nodules (5-25 mm) with cavitation in computed tomography (CT scan) from 6 months ago. More clinical evaluation and imaging techniques showed that her liver and spleen were also involved. The infection of the patient with hydatid cyst was confirmed by ELISA and albendazole was used to treat. During recent hospitalization she was under treatment with meropenem and levofloxacin due to fever and productive cough. The symptoms and fever was improved within two weeks.

Conclusion: As respect, the importance of hydatid cyst in Iran, it is necessary to pay more attention to complicated and disseminated hydatid cyst in immune- deficient/ suppressed patients.

Keywords: hydatid cyst, multiple organs, Baqiyatallah hospital

Molecular and morphological study of *Galba schirazensis* from southeastern Iran (Pulmonata, Lymnaeidae)

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Introduction and aims: Snails of the family Lymnaeidae are the focus of numerous studies are intermediate hosts of fascioliasis. *Galba truncatula* and *G. schirazensis* have high phenotypic similarity and genotypic differences and this similarity detection is difficult with conchological methods. We differentiated *G. schirazensis* from *G. truncatula* by using molecular tools in southeastern Iran.

Material and methods: Snails were collected by hand from Giroft, Bam and Faryab counties in south of Kerman province. Following morphological examination according to taxonomic keys a piece of foot was dissected and frozen at -20°C. DNA extraction was carried out and mitochondrial CO1 gene fragment was amplified using a universal primer and finally sequenced by commercial sequencing. Sequences were aligned and Phylogenetic analysis done using BioEdit and MEGA 6.0 softwares respectively. Also *F. hepatica* infection of the snails were detected using PCR, amplification of a 124 bp non-coding tandem repeat found in *Fasciola* genome

Results: In present study we collected an average of 20 snails from each location with 4-5 whorl and shell length size (3.02 - 6.81, \bar{x} = 4.91). Two cox1 haplotypes (KT267209-KT267212) were placed in two different sister clades including specimens from Bam together with *G. schirazensis* isolates from Peru and the Faryab and Jiroft samples located in the same clade with Iranian isolates from north. Molecular investigation of *F. hepatica* DNA has not detected the parasite in any of *G. schirazensis* specimens.

Conclusion

G. schirazensis is capable have sympatric distribution with *G. truncatula* and The use of molecular methods together with morphological data provides us with a more comprehensive picture of snail taxonomy. However discussed about causal ability of *G. schirazensis* in fasciolosis transmitting.

Key words : *Galba schirazensis*, *Galba truncatula*, CO1, Phylogenetic

First report of Amphistomiasis due to *Explanatum explanatum* in cattle, from Kerman province, Iran

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Introduction and aims: Paramphistomiasis caused by different species of trematodes in the family paramphistomatidae is one of the neglected parasitic diseases of livestock. Adult stage of the paramphistomes are seen in the digestive system of livestock. Adult stages are generally nonpathogenic for their hosts, but immature stages migrating to the anterior small intestinal mucosa can cause severe pathogenesis including enteritis, hemorrhage, anorexia, severe diarrhea, and even mortality. Several species of paramphistomes have been reported in Iran, however little is known about the frequency of *Explanatum explanatum* in the cattle bile ducts. This study is the first report of *E. explanatum* in Kerman Province.

Materials and methods: The worm specimen was collected from the liver of the native cattle from the Kerman abattoir, southeastern Iran. Recovered trematodes were rinsed in saline and fixed in 70% ethanol. The flukes were stained with Carmine and permanently mounted in Canada balsam. Measurements of morphological features including length, width, posterior sucker diameter, were made with a calibrated optical microscope.

Results: During the abattoir inspection of cattle, a large number of parasites were seen in bile ducts of the liver of a native cattle. Using taxonomic identification keys the worms were identified as *E. explanatum*.

Discussion: This trematode has been reported from Iraq and Afghanistan. It is worth noting that during recent surveys in Iran, *E. explanatum* has been identified in livestock from other regions of the country. Specific epidemiological studies with exact slaughter inspections are needed to recognize the extent of the parasite prevalence in Iran.

Keywords: *Explanatum explanatum*, Kerman, livestock, paramphistome

Anti-Toxoplasma effect of the Moringa (*Moringa oleifera*) and Milk thistle(*Silybum marianum*) extracts

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Background: *Toxoplasma gondii* is an obligate intracellular parasitic that cause serious contamination in humans and warm-blooded animals. The currently available agents for use against Toxoplasmosis have serious limitations, thus the aim of the present study was to investigate the Anti-Toxoplasma *gondii* activities of *Moringa oleifera* and *Silybum marianum* extracts.

Materials and methods: Virulent RH strain of *T. gondii* was maintained in mice and macrophages containing tachyzoites were aspirated from the peritoneal cavity. Tachyzoites were incubated with the hydroalcoholic extract of *M. oleifera* and the aqueous extract of *S. marianum* (5-200 µg/ml) for 10, 30, 60, 120 and 180 minutes and the viability of tachyzoites were evaluated by trypan blue staining. Also, Vero cell pre-treated with extracts and then infected with *T. gondii* and metabolic activity and cytotoxicity of cells assessed using MTT method.

Results: *M. oleifera* extracts killed *T. gondii* tachyzoites after 120 minutes in dose depend manner and *S. marianum* had no direct effect on tachyzoites during test time. In MTT assay, infected Vero cell metabolized MTT more than non-infected cells (control) and only *S. marianum* could decrease MTT to control level in dose dependence manner.

Conclusion. Our results indicating that *M. oleifera* had more parasitocidal potency than *S. marianum* against *T. gondii* tachyzoites but was toxic to host cell in concentration above 5 µg/ml. *S. marianum* had no effect on tachyzoites directly but can protect host cell and might inhibiting penetration of tachyzoites into cells by stabilizing cell membrane.

Keywords: *Toxoplasma gondii*, *Moringa oleifera*, *Silybum marianum*, MTT method

Some experimental skills in slide preparation at sandflies morphological studies

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Objectives: Good slide preparation is many important in morphology and morphometric studies at phlebotomine sandflies. Routinely, 18×18mm cover glass and fixative are used for sample mounting.

Materials and Methods: In the current study, small cover glass (4×4mm) and Pouri's medium as a fixative agent have been used in slide preparation. About 3000 slides of 20 species of sandflies have been prepared using this method.

Results: Based on our experience in this study, the application of small slide glasses will be beneficial, economic and faster. In this experience immediately after mounting it could be placed in collection boxes especially in field conditions. This method can decrease the cost of study about 10% by reducing consumed materials such as slides, cover glasses, fixative, and boxes. 10 specimens could be mounted in each slide in average, it will decrease the number of collection boxes used for slide collection.

Discussion: This experience could be applicable in other scientific studies. The detailed experiences will be discussed in the presentation.

Keywords: Slide, Cover Glass, Cost Effectiveness, Entomology, Experience

An invented method for isolation of *Leishmania* parasites DNA from infected sandflies

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Objectives: Nowadays detection and identification of the *Leishmania* species are doing by molecular techniques. So, DNA extraction of *Leishmania* is the main step.

Materials and Methods: This study carried out to extract *Leishmania* parasites DNA that was found in the sand flies. The specimens collected by the sticky and light traps. A100 microliters of sterile water added in each microtubes containing sandflies body (without head and genitalia) after morphological identification. The supernatant transferred into another microtube for further DNA extraction. *Leishmania* parasites crashed by using a sterile pestle in single or pooled samples.

Results: In the current study using this method, *Leishmania major*, *Leishmania tropica*, *Leishmania infantum*, and *Leishmania donovani* parasites were detected in *Phlebotomus alexandri* sandflies collected from western Iran.

Discussion: Using this method, the remaining particles of the sample will be protected both parasites and vector DNA without destroying in room temperature.

Keywords: Sandflies, *Leishmania*, DNA extraction, Parasites, Iran

Evaluation of anti-Leishmaniasis effect of hydroalcoholic extract of *Ganoderma leucidum* on *Leishmania major* parasites in vitro

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Introduction and objectives: *Leishmania major* is the causative agent of cutaneous Leishmaniasis in Iran. Because of the side effects of current drugs and the emergence of drug resistance in some areas, researchers have been seeking more effective and non-complicated compounds, especially herbal medicines for the treatment of Leishmaniasis. The aim of this study was to evaluate the anti-Leishmanial effect of *Ganoderma leucidum* mushroom.

Methods: Iranian *Leishmania major* parasite species (MRHO/IR/75/ER) was cultured in Novy-Mac Neal-Nicolle (NNN) and Roswell Park Memorial Institute-1640 (RPMI)-enriched media, and in the metacyclic phase transferred to a 96-well plate at the time of preparation of the hydroalcoholic extracts. Then, concentrations of 10, 50, 100, 150, and 200 µg/ml were used at 24, 48, and 72 hours. The amount of live promastigotes was assessed by using a hemocytometer and MTT colorimetric assay. Data were analyzed using Kolmogorov-Simonov, ANOVA, and repeated measures ANOVA tests, and $P < 0.05$ was considered as a meaningful level.

Results: The hydroalcoholic extract of this mushroom at high concentrations (150 and 200 µg/ml) inhibited the growth of the parasite significantly, and had a direct correlation with the increase of time ($P < 0.001$). Moreover, in the MTT method, it was a significant difference in the mean of optical absorption of different extract concentrations at 24, 48, and 72 hours ($P < 0.001$).

Discussion and conclusion: The observed antileishmanial effect of *Ganoderma leucidum* extract on the number and growth of live promastigotes at high concentrations can be resulted due to its compounds such as tannins, flavonoids, triterophenides, and polysaccharides. It requires further research on the purification of its compounds and work on the animal model of Leishmaniasis.

Keywords: Cutaneous Leishmaniasis; *Ganoderma leucidum*; MTT

Survey of *Sarcocystis* as a hidden health problem in Iran

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Sarcocystis is one of the parasitic protozoa which more than 130 species have been identified. This protozoan was first isolated from the rat muscles in 1843. Sporocysts ingested by the intermediate host (cattle for *Sarcocystis hominis*) after ingestion of oocysts. In Iran, the contamination of domestic and farm animals with this parasite is high according to the results of epidemiological studies. In *sarcocystosis*, rice-like shape cysts are replaced in the muscles of ruminants and humans are infected by eating raw or undercooked meat. Also, Humans may become dead-end hosts for non-human *Sarcocystis* spp. after the accidental ingestion of oocysts. This parasite causes asthma, allergies and meningitis in humans by producing a toxin called sarcocystin. It can also cause abortion in humans. Rice-like shape cysts may not be seen during meat inspection, which may spread the disease. In addition to the damage caused by the parasitic disease, this parasite also causes many economic losses. In order to prevent economic losses, hygiene control and nutrition enhancement, health inspections as well as nutritional recommendations should be strictly implemented.

Keywords: Food safety, Public health, *Sarcocyst*

Assessment of *Plasmodium berghei* exflagellation rate correlation with day hours

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Introduction: *Plasmodium* species cause approximately half a million deaths all around the world each year. The exflagellation of microgametocytes which takes place in the stomach of the Anopheles mosquito has crucial role in parasites life cycle and detection of environmental factors affecting the dynamics of exflagellation could help us to develop new methods of preventing the disease.

Methods: In this research, we evaluated exflagellation rate in term of daily hours. Three days after injection of parasites into balb/c mice, the number of exflagellation recorded every 3 hours in 4 consecutive days. Examination group consisted of 10 Balb/C mice which under gone evaluation in the optimal thermal condition (20 centigrade of Celsius). In order to achieve a tangible result, the mean value of exflagellation in 5 different microscopic fields (X40) of every blood sample collected from tail of each mouse was calculated 20 minutes after adding cold culture medium.

Results: Analysis of the results revealed considerable fluctuations throughout the examination. To be more exact, the highest rate of exflagellation was recorded at noon (11AM) while on the other hand, early hours of the night (around 20) is likely the time that parasite has its least tendency to exflagellate. In addition, number of exflagellations experienced major decrementation after the 5th day of injection and this trend continued to the end.

Conclusion: It was suggested that daily cycle have effect on *plasmodium* species exflagellation inside mice bodies. In our test, daytime was the most appropriate time for parasite to exflagellate which is in contradiction with mosquito blood feeding pattern in wild that prefer nights to feed on blood. It seems that registered variations may have roots in keeping and blood feeding habits in laboratory and insectarium environment that feeding anopheles mosquitos takes place mostly in morning. The number of exflagellations decrease probably is related to whole blood volume reduction due to multiple blood sample collection from mouse and immune system activation against parasite which can prevent exflagellation.

Keywords: *Plasmodium berghei*, Exflagellation, Day Hours, Anopheles

Molecular assessment of *Toxoplasma gondii* in the magpie (*Pica pica*) and poultry in Isfahan, Iran

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Background: Felines are definitive hosts of *Toxoplasma gondii* and many warm-blooded animals including birds are serving as intermediated hosts. Birds can be infected with *T. gondii* through ingestion of oocysts from the contaminated environments by feline feces. We know little about the prevalence of *T. gondii* in crows and domestic chicken (*Gallus gallus*). Hence, the aim of this study was to determine the presence of *T. gondii* in the magpie (*Pica pica*) and poultry by a molecular method in Isfahan, central Iran.

Material and methods: A total of 13 brain samples of a magpie and 32 brain samples of chicken were collected from different parts of Isfahan in 2019 year. Individual brain samples of the hosts were homogenized and DNA was extracted using a phenol-chloroform extraction method. The 344 bp fragment of GRA6 gene was amplified using nested-polymerase chain reaction (PCR) method for evaluating the presence of *T. gondii* DNA.

Results: The results showed that 8 (61.53%) out of 13 magpie samples and 22 (68.75%) out of 32 avian samples, had *T. gondii* DNA.

Conclusion: Based on the results of the present study, magpie and domestic chicken and probably other birds have *T. gondii* in their tissues and may have a significant role in the transmission of the parasites to human and even felines.

Keywords: *Toxoplasma gondii*, nested-PCR, GRA6, Magpie, Bird

Detection of *Toxoplasma gondii* in the sheep meat in Isfahan using GRA6 gene

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Background: Toxoplasmosis is a prevalent infection present all over the globe and a major public health concern for humans and domestic animals. It is caused by the parasitic protozoan *Toxoplasma gondii*. The infection may cause serious reproductive problems and economic losses to the sheep industry all over the world. *Toxoplasma gondii* has been recognized as a significant cause of lambing loss. The aim of the present study was to determine the frequency *T. gondii* infection in sheep using a nested polymerase chain reaction (PCR) by targeting the T. gondii GRA6 gene.

Material and methods: Biological specimens were collected from 30 hearts, 52 esophagi, 44 diaphragms and 60 brains from slathered sheep in Isfahan abattoir in central Iran. DNA was extracted with the phenol-chloroform method. The 344 bp fragment of GRA6 gene was amplified using nested-PCR method for evaluating the presence of *T. gondii* DNA.

Results: Sixteen (53.33%) out of 30 heart samples, 18 (34.61%) out of 52 esophagus samples, 11 (25%) out of 44 diaphragm samples, and 33 (55%) out of 60 brain samples had *T. gondii* DNA.

Conclusion: Based on the results of the present study, **the highest frequency of *T. gondii* infection was observed in the brain samples of the studied sheep.** Nevertheless, the high rate of *T. gondii* infection in sheep, the host that may play a major role in human Toxoplasmosis, cannot be ignored in the Isfahan area.

Keywords: *Toxoplasma gondii*, nested-PCR, GRA6, Sheep

Prevalence of anti-*Toxoplasma* IgM and IgG seropositivity in patients referred to a clinical laboratory in Urmia, Iran

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Introduction: *Toxoplasma gondii* is a cosmopolitan protozoan, infecting a wide variety of hosts, including humans. It is one of the most prevalent parasitic infections in Iran. The present study aimed to determine the prevalence of anti-*Toxoplasma* IgM and IgG seropositivity in patients referred to a clinical laboratory in Urmia, northwest of Iran.

Materials and Methods: In the present retrospective study, the test results of 471 (80 males [17%] and 391 females [83%]) patients referred to a clinical laboratory in Urmia with *T. gondii* antibodies (IgM and IgG) test from March 2017 to July 2019 were collected and studied. The anti-*Toxoplasma* IgM and IgG test have been done by the enzyme-linked immunosorbent assay (ELISA).

Results: Based on the results, 10 (2.55%) females and three (3.75%) males showed positive titer of anti-*Toxoplasma* IgM in their serum. Furthermore, the anti-*Toxoplasma* IgG test results of the patients demonstrated that 108 (27.62%) females and 18 (22.5%) males had positive antibody titers in their serum. Besides, 11 (2.33%) of the tested patients were positive for both tested antibodies, and also two (0.42%) were negative for IgG yet confirmed to be positive for IgM.

Conclusion: Detection of *Toxoplasma* infection is particularly important, especially in pregnant women because of the risk of congenital Toxoplasmosis. According to the results of the present study, the prevalence of the infection is similar to the reports from neighboring regions; however, nearly 4% of the studied patients were positive for anti-*Toxoplasma* IgM and considering that the majority of the studied population were females, even low frequency of the possible acute infection cannot be ignored.

Keywords: ELISA, IgG, IgM, *Toxoplasma*, Urmia, Iran

Current status of intestinal parasitic infections and associated risk factors in rural population of Guilan province, northern Iran: Trichostrongylosis the most prevalent helminthic infection

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Background and objectives: Intestinal parasitic infections are still considered as a major health hazard in human communities with poor economy and sanitation in particular. This study aimed to determine the distribution of enteric parasitic infections and related risk factors among rural communities of Guilan province, Northern Iran.

Methodology: This cross-sectional study was performed in rural areas of Masal and Shanderman district from February 2018 to December 2018. A total of 917 stool samples were collected and examined for presence of intestinal helminthes and protozoa using direct, formalin-ether and Kato-Katz techniques.

Results: A total of 156 (17%) out of 917 examined individuals were infected with intestinal parasites. The overall prevalence of protozoan, helminthic and mixed infections were 11.8% (108/917), 4.5% (41/917) and 0.8% (7/917) respectively. *Blastocystis hominis* was the most prevalent intestinal protozoa (9.6%) followed by *Giardia lamblia* (1.9%), *Endolimax nana* (1.1%), *E. coli* (0.8%) and *E. hartmani* (0.1%). The highest prevalence of intestinal helminthes belonged to *Trichostrongylus* spp. (3.5%) followed by *Strongyloides stercoralis* (1.3%). Statistical analysis showed significant correlation between giardiasis and sex ($P < 0.03$) but not by age and drinking water source. On the other hand, prevalence of enteric helminthes was influenced by close contact with livestock, keeping herbivorous animals at home, job, education and eating uncooked vegetables.

Discussion and conclusion: Results of the present study indicate a decreasing trend in prevalence of intestinal parasitic infections in Guilan province in comparison to the past few decades. Hook worm infections which was previously very prevalent in the area was absent while trichostrongylosis showed a high prevalence in rural residents of the studies area.

Keywords: Intestinal parasites, Risk factors, Guilan, Iran

Prevalence of *Fasciola hepatica* in Slaughtered cattles in Maku Free Zone

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Background: Fasciolosis refers to an infection caused by human and animal infections with feces of the genus *Fasciola* (*Fasciola hepatica* and *Fasciola gigantica*). *Fasciola* parasites are tertiary-worm-worms that are the natural parasite of ruminating animals, including sheep, goat, cattle, buffalo, pigs, camels.

Methods: This study was carried out descriptively and prospectively concerning the slaughter chart of the years 1397 and 1398 available in maku industrial slaughterhouse and the veterinary office of the maku. The information present in the charts were transferred to the tables designed according to desired variables such as the kind and the number of the slaughtered domestic animals, the kind of the parasite, the number of the infected domestic animals, month, season and the year.

Results: the prevalence of *Fasciola hepatica* was In this study Of the 1200 cattles slaughtered in the Maku slaughterhouse, 80 vertebrae had liver infected with *Fasciola hepatica*. Pollution in winter was 13% more than in other seasons.

Keywords: *Fasciola hepatica*, Infection, Slaughterhouse.

Study the effect mix of *Peganum harmala* & Lavender plantes on the death of *Pediculus humanus*

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Abstract

Background: Lice is an external parasite that dates back to prehistoric times. Lice feeds on human blood and its saliva contains anticoagulants and prevents blood clots and causes severe itching. A 2011 study showed that lavender oil helps prevent head lice growth and can even cure it.

Methods: In this study, 110 infected people were examined. The age group was between 9 years old and 40 years old. The spin extract and its combination with lavender were used.

Results: The dilutions used were 100, 50, 25, 10 percent, respectively. The death rate in adult lice at a concentration of 100% over a three-day use period, was 93%. This study shows Herbal compounds can be replaced by chemical compounds in the treatment of lice.

Keywords : Concentration, Treatment, *Peganum harmala*, *Pediculus humanus*

Inoculation of Exposed and Non-exposed Protoscoleces to Protoscolicidal Agents in to Peritoneal Cavity of Balb/C Mice: A Histopathological Evaluation of Author :Experimental Hydatidosis

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Introduction: Choice treatment of hydatid cyst is surgery and it is necessary to use protoscolicidal agents having strong protoscolicidal effect and the least side effects. In this regard, application of experimental in vivo methods than in vitro methods for evaluation of the extent of protoscolicidal effect is very useful. The purpose of this study is histopathological evaluation of Balb/C peritoneum after the inoculation of protoscoleces which have been exposed to garlic chloroformic extract and normal saline at three different times of exposure.

Material and Method: In order to evaluate the protoscolicidal effect of garlic chloroformic extract, protoscoleces were exposed to this agent along with positive control cetrime 0.5%, negative control normal saline and two solutions of silver nitrate 0.5% and hypertonic sodium chloride 20% at different temperature and different times of exposure including 1, 2 and 5 minutes followed by inoculation in to Balb/C peritoneum (5 mice in each group). After 8 months, mice were euthanized and necropsied for checking any possible hydatid cyst in their peritoneal cavity. Macroscopic and microscopic evaluation including histopathological examinations were made from the cysts.

Results: In examining the abdominal area of the mice, tissues suspected to cyst were separated, fixed in formalin 10%. Specific sections from each sample and paraffin embedded blocks were provided and then, histopathological slides were prepared and stained. In all cases that hydatid cyst have been detected by macroscopic method, the detection was also confirmed by histopathological slides. Slides related to cetrime and hypertonic sodium chloride groups were all negative. In chloroformic extract group, there was only one suspected mice but the observed histopathological slide was negative. In silver nitrate groups, 2 and 3 mice were suspected and slides were reported as positive. Also in normal saline groups, there were 2, 2 and 3 suspected mice which histopathological slides were reported to be positive and confirmed. In most of the positive slides, in addition to cyst, inflammation together with hyperemia in cyst adjacent tissues were also detected. Such tissues were necrotic and calcified. No protoscoleces were observed in the cysts.

Conclusion: Our results revealed that application of experimental models and using histopathology as an appropriate confirmation method for hydatid cysts is suitable method and could be used for evaluation of protoscolicidal agents or even drug evaluation.

Keywords: Hydatid Cyst, protoscolicidal Agent, Histopathology

A novel unlabeled probe based real time PCR and modified semi nested PCR –sequencing as molecular tools for analysis of chloroquine resistant associated genes (*pvmdr1* and *pvcrt-o*) in *Plasmodium vivax* isolates from Afghanistan.

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Introduction: Chloroquine (CQ) resistance *Plasmodium vivax* isolates have been reported from many endemic regions in the world. The *pvmdr-1* and *pvcrt-o* (K10 insertion) genes are the possible markers of CQ-resistance *P. vivax* isolates. In this work, we aimed to evaluate the prevalence of mutations in the *pvmdr-1* and K10 insertion in the *pvcrt-o* genes.

Material and methods: *P. vivax* isolates were collected from Laghman, Baghlan and Khost provinces. For investigation of polymorphisms of desired regions in *pvmdr-1* and *pvcrt-o* genes, sequencing was applied on the PCR products. We developed a new asymmetric qPCR and melting analysis assay based on unlabeled probe for scanning of K10 insertion in *pvcrt-o* gene.

Results: The analysis of sequencing data of the *pvmdr-1* gene showed wild type Y976 and K997 and mutant M958 and L1076 in all 33 isolates from three provinces and submitted in GenBank.

Of 36 samples that evaluated for K10 insertion in *pvcrt-o*, 2/18(11%), 0/10(0%) and 0/8(0%) isolates from Laghman, Baghlan and Khost province possessed K10 insertion, respectively, that confirmed by either sequencing and unlabeled probes and submitted in GenBank.

Conclusion: The existence of 2 samples with K10 insertion and 33 samples with *pvmdr1* polymorphism indicating on CQ resistance *P. vivax* populations in Afghanistan. This can lead to spreading of resistance strains in the society. Furthermore, unlabeled probes are simple and inexpensive alternative tools for mutations scanning. This study promoted the scanning of *pvcrt-o* K10 insertion with a modified semi nested PCR and subsequent sequencing for the first time.

Keywords: *Plasmodium vivax*, Afghanistan, *pvmdr-1*, *pvcrt-o*, Chloroquine resistance.

The effect of glucose on *Echinococcusgranulosus*microcyst development, *in vitro*

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Background and objectives:Hydatid microcyst developed in culture medium have been used for assessment of *Echinococcusgranulosus*larva susceptibility to different compounds; but this procedure is time consuming and risky. Glucose is one of the most important factors which have main roles in development of *E.granulosus*. So, present study was designed to evaluate different level of glucose on microcysts development *in vitro*.

Methodology: Protoscoleces was aspirated from liver hydatid cyst of infected sheep and maintained in the RPMI₁₆₄₀ culture medium containing 20% (v/v) fetal calf serum (FCS), and different concentrations of glucose (2, 6 and 8 mg/ml). RPMI₁₆₄₀ medium contain 4 mg/ml of glucose was served as a control. Development factors such as beginning of vesiculation, appearance of laminated layer, appearance and percent of developed microcysts were followed using inverted microscope daily.

Results:Larger microcysts in shorter time were achieved from culture medium with 6mg/ml glucose (820×640 μm) than microcysts from control group (450×380 μm), but the density and number of developed microcysts was fewer than control group.

Discussion and conclusion: Present study showed glucose could affect development of *E.granulosus* *in vitro*. Another study carried out on insulin (as an affecting hormone on glucose uptake) has shown the same results; but more studies should be done on utilization and effectiveness of optimal glucose concentration in *in vitro* condition.

Keywords: *Echinococcusgranulosus*, glucose, development, microcyst, *in vitro*

Survey of Hydatid Cyst in Sheep and Goats Slaughtered in the Tabriz Industrial Slaughterhouse

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Background and Objectives: Echinococcosis or hydatosis is an oonotic disease which caused by larval stages of 2-7 mm long tapeworm *Echinococcus granulosus* that inhabits the intestinal tract of dog and other carnivores. All domesticated animals can be invaded by the larvae of the parasite. Infection occurs by ingestion of water or food contaminated with eggs of the tapeworm.

Methods: In this study which was performed in winter of 2018 and spring of 2019 in slaughterhouse of Tabriz, internal organs of 23817 sheep and 3381 goats including lungs and liver were inspected macroscopically.

Results: Hydatid cyst was detected in lung and liver of sheep with the amount of 6/62%, 4/91% and for goats was 3/04% and 2/77%, respectively. The rate of infection in the lung and liver of sheep was 5/77% and 3/47% and for goat was 3/4%, 2/86% in spring. The rate of infection in the lung and liver of sheep was 7/17%, 5/74% and in goat was 3/32%, 2/38 in winter, respectively.

Conclusions: In this study, similar to most other studies the rate of infection in the goat is lower than sheep and in general, in both goat and sheep, lung infection was greater than liver. According to this study, to reduce the percentage of hydatid cyst infection, a regular survey of the prevalence of the disease for preventive planning in the livestock population of the region is necessary.

Keywords: Hydatid cyst, Tabriz, Echinococcosis

A Twenty- Four Years Study on the Malaria Trend in Southwestern Iran (1995-2018)

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Abstract

Aim & Background: Almost all malaria transmission happens in the southeastern regions of the Iran. In Iran, there is a significant decrease in disease burden; however, the overall trend of malaria prevalence is not investigated or well-documented in different localities. Hence, this study is aimed to investigate the epidemiologic features of malaria cases in Gotvand County from 1995 until 2018.

Material and methods: This descriptive cross-sectional survey investigates malaria-related factors during a 24-year period of time based on existing data and information extracted at Gotvand's Health Services Center during 1995-2018.

Results: The highest (23, 5 and 5) cases of malaria were occurred in 1995, 1996 and 2006, respectively. The majority of cases (67.4%) were male. Most cases of malaria were due to *P. vivax* (97.8%) followed by *P. falciparum* (2.2%).

Discussion & Conclusion: As it was expected, *P. vivax* accounted for the majority of parasites, which is consistent with results from other studies in other parts of the world. In recent years, the incidence rate of *P. falciparum* infection has significantly reduced. This reduction versus the increased prevalence of *P. vivax* in a region indicates an increase in local transmission of malaria. Due to the specific climatic status in Gotvand County, the optimum circumstances exist for the growth of mosquitoes as the insect's malaria vectors; thus, the risk of malaria epidemics should be considered constantly.

Keywords: Malaria, Epidemiology, Trend, Prevalence, Iran.

Seroepidemiological Evaluation of Hydatidosis in Julfa (North West Iran) 2018

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Introduction: The hydatid cyst is a larval stage of *Echinococcus granulosus*, causing hydatidosis in hosts such as herbivores and humans. Due to the lack of a seroepidemiological study in Julfa city, located in North of West Azarbaijan (Iran), this study was conducted to evaluate the prevalence of hydatid cyst in residents of urban and rural areas of Julfa city 2017-2018.

Materials and Methods: In this descriptive cross-sectional study, a sample of 1296 people aged 5-75 years old from urban and rural areas of Julfa city were selected. About 7 cc of blood samples were taken from each person. Indirect Haemagglutination Test was performed and Elisa was performed for serum positive people. The results were analyzed by descriptive statistics tests.

Results: From a total of 1296 individuals in this study who were examined for hydatid cyst by IHA test, the test result of 25 people was positive (1.92%). From the people who had a negative IHA test result, a blood sample of 269 people, as well as a blood sample of 25 people who were positive for their test, were tested for the hydatid cyst by ELISA. Fourteen people from the subjects tested had a false positive result, accounting for a total of 283 negative test results with an ELISA test. The false positive rate has been 4.9% in this study. The sensitivity of IHA test was 100% in this study.

Conclusion: Research has shown that rate infection in the area is not high; however, it is important to pay attention to the at-risk people, especially women, and to take effective preventive measures in coordination with all organizations especially in the field of public health and control of dogs in the region in order to prevent health and economic consequences.

Keywords: Seroepidemiology, Hydatidosis, Julfa city, Iran

Evaluation of oxidative stress indices of the liver of the cows infected with *Dicrocoelium dendriticum* Parasite and its relation with the severity of parasitic infection

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Background: The aim of this study was to investigate the oxidative indicators of cow livers infected with *Dicrocoelium dendriticum* and its relation with the severity of parasitic infection.

Methods: During January to March 2018, nineteen liver of cows infected with *Dicrocoelium dendriticum* and fifteen healthy liver cows without any macroscopic lesions were collected from the Mashhad industrial slaughterhouse. To evaluate oxidative stress indices four tissue samples were taken from each liver. Samples stored in a freezer at a temperature of -20°C. Oxidative stress indices including malondialdehyde, glutathione reductase and total antioxidant capacity were measured by FRAP method in homogenized tissue livers. Then, the liver tissues were sent to the parasitology laboratory to measure the parasitic infection severity.

Results: According to the results, the GSH level in the infected group was significantly lower than the healthy group, but in the FRAP and MDA tests there were no significant differences between the healthy and infected groups. The severity of parasitic infection varied from 48 to 1656. In this study there was no correlation between oxidative stress indices and the severity of parasitic infection.

Conclusion: According to the results it seems that liver damage caused by oxidative stress is not so effective on liver damage caused by *Dicrocoelium dendriticum*. In general, as the *Dicrocoelium dendriticum* does not have liver migration, damage to the liver parenchyma is low. It seems that histopathological lesions of the parasite are because of its mechanical trauma and toxic productions of trematode.

Keywords: *Dicrocoelium dendriticum*, oxidative stress, cow liver, Parasite severity

Survey of Hydatid Cyst in Sheep and Goats Slaughtered in the Tabriz Industrial Slaughterhouse

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Background and Objectives: Echinococcosis or hydatosis is a zoonotic disease which is caused by larval stages of 2-7 mm long tapeworm *Echinococcus granulosus* that inhabits the intestinal tract of dog and other carnivores. All domesticated animals can be invaded by the larvae of the parasite. Infection occurs by ingestion of water or food contaminated with eggs of the tapeworm.

Methods: In this study which was performed in winter of 2018 and spring of 2019 in slaughterhouse of Tabriz, internal organs of 23817 sheep and 3381 goats including lungs and liver were inspected macroscopically.

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Conclusions: In this study, similar to most other studies the rate of infection in the goat is lower than sheep and in general, in both goat and sheep, lung infection was greater than liver. According to this study, to reduce the percentage of hydatid cyst infection, a regular survey of the prevalence of the disease for preventive planning in the livestock population of the region is necessary.

Keywords: Hydatid cyst, Tabriz, Echinococcosis

The effect of glucose on *Echinococcusgranulosus*microcyst development, *in vitro*

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Background and objectives: Hydatid microcyst developed in culture medium have been used for assessment of *Echinococcusgranulosus*larva susceptibility to different compounds; but this procedure is time consuming and risky. Glucose is one of the most important factors which have main roles in development of *E.granulosus*. So, present study was designed to evaluate different level of glucose on microcysts development *in vitro*.

Methodology: Protoscoleces was aspirated from liver hydatid cyst of infected sheep and maintained in the RPMI₁₆₄₀ culture medium containing 20% (v/v) fetal calf serum (FCS), and different concentrations of glucose (2, 6 and 8 mg/ml). RPMI₁₆₄₀ medium contain 4 mg/ml of glucose was served as a control. Development factors such as beginning of vesiculation, appearance of laminated layer, appearance and percent of developed microcysts were followed using inverted microscope daily.

Results: Larger microcysts in shorter time were achieved from culture medium with 6mg/ml glucose (820×640 μm) than microcysts from control group (450×380 μm), but the density and number of developed microcysts was fewer than control group.

Discussion and conclusion: Present study showed glucose could affect development of *E.granulosus* *in vitro*. Another study carried out on insulin (as an affecting hormone on glucose uptake) has shown the same results; but more studies should be done on utilization and effectiveness of optimal glucose concentration in *in vitro* condition.

Keywords: *Echinococcusgranulosus*, glucose, development, microcyst, *in vitro*

QSAR modeling, molecular docking and molecular dynamic simulations studies of novel scaffold of thiazolidin-4-one derivatives as anti-*Toxoplasma gondii* agents

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Introduction

Toxoplasma gondii is an obligate intracellular protozoa that can infect a wide variety of warm-blooded animals and humans. It was claimed that novel anti-*Toxoplasma gondii* agents were optimized as potential drug candidates, designed and created as significant agents.

Methods

In this work, molecular modeling studies including CoMFA, CoMFA-RF, CoMSIA and HQSAR were performed on a series of thiazolidin-4-one derivatives as anti-*T.gondii* agents. The statistical qualities of generated models were justified by internal and external validation i.e., cross-validated correlation coefficient (q^2), non-cross-validated correlation coefficient (r_{ncv}^2) and predicted correlation coefficient (r_{pred}^2), respectively.

Results

The CoMFA (q^2 , 0.897; r_{ncv}^2 , 0.933; r_{pred}^2 , 0.938), CoMFA-RF (q^2 , 0.900; r_{ncv}^2 , 0.935; r_{pred}^2 , 0.998), CoMSIA (q^2 , 0.910; r_{ncv}^2 , 0.950; r_{pred}^2 , 0.998) and HQSAR models (q^2 , 0.924; r_{ncv}^2 , 0.953; r_{pred}^2 , 0.995) for training and test set yielded significant statistical results.

Conclusion

Therefore, these QSAR models were excellent, robust and had good predictive capability. Contour maps of the QSAR models were generated and validated by molecular dynamic simulation-assisted molecular docking study. The final QSAR models could be useful for design and development of novel potent anti-*T. gondii* agents.

Keyword: *Toxoplasma gondii*; 3D-QSAR, HQSAR, thiazolidin-4-one derivatives, molecular dynamic simulations

Strongyloidiasis in Immunocompromised Hosts

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Introduction: *Strongyloides stercoralis* is an intestinal nematode endemic in the tropics and subtropics where hygiene standards and sanitary conditions are deficient. Infection in the immunocompetent host is usually associated with mild gastrointestinal symptoms.

This soil-transmitted helminth is the only parasite that secretes larvae in feces and alternates between free-living cycles (rhabditiform larvae) and parasitic cycles (filariform infective larvae). This review study focuses on describing *Strongyloides* infection in the immunocompromised host, including immune response against this infection.

Methodology: In this review study several databases including Pubmed, Elsevier- Scindirect, Google scholar, Scopus have been searched.

Result: Primary goal in organ transplantation is the prevention or effective treatment of infection, the most common life-threatening complication is long-term immunosuppressive therapy.

The challenges involved in achieving this goal are several: a broad range of potential sources of infection ranging from latent viruses to pathogens of both community and hospital origin infections.

Immunocompetent hosts typically are asymptomatic, despite chronic *Strongyloides* infection. In contrast, immunocompromised patients are at risk for hyper infection syndrome and disseminated disease, with a fatality rate of more than 50%.

Discussion and conclusion: There are small endemic foci of the disease in countries with a temperate climate. In immunocompetent individuals, this infection is often asymptomatic or has a mild symptomatology, characterized by high eosinophilia, pulmonary symptoms, dermatological manifestations, and intestinal symptoms. However, there are some risk factors, such as co-infection with HIV or human T-cell lympho-tropic virus (HTLV1).

Diagnosis is often delayed due to the nonspecific nature of presenting symptoms, and early diagnosis relies on a high index of clinical suspicion to prevent death.

However, newer diagnostic modalities, such as RT-PCR can be an effective tool for the early detection of transmission among solid organ transplant recipients.

Effects of combination usage Dextran and a new feed additive on CFR at laying Farms Involved by Dermanyssidae mite

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Introduction & Objective: Mites Family Dermanyssidae the most important economic problem is the laying of poultry farms be. Mites with painful bruises and blood pressure can cause stress and decrease seriously egg production. The occurrence of drug resistance against toxins and the problem of chemical pesticide residues has led to Recent studies have focused on the consumption of various herbs, especially their extracts in water Consumption or powder of plants in a Consuming. The purpose of this study Effects of combination usage Dextran and a new feed additive on CFR at laying Farms Involved by Dermanyssidae mite.

Material and method: In the present study, mayto-fade Plus mineral and herbal supplement is used alone and in conjunction with periotic Mito with Dextran in a ration of chicken laying on a farm And its anti-mite were evaluated individually and combined. The crowds of mites are trapped with special traps before starting to take supplements And subsequently measured over a period of 8 consecutive weeks.

Result: According to the results, the consumption of 1 kg / ton MF+ supplement alone could reduce the population of mites from 62.2 to 12.7. This decrease in Mite population was higher in combination with MF + the Mito (dextran) periotic complement, showing from 64.6 to 10.2. But consuming the Mito supplement alone (dextran) did not show a significant reduction. Effect level MF + supplemented therapy with a single dose of 1 kg /ton alone was 79.58% However, the combined effect of MF + with dextran was 84.21%.

Discussion and conclusion: the therapeutic effect of MF + alone was almost acceptable but because The amount of initial contamination of the farm was very high, this value can be very well evaluated. Using supplements such as Mito can enhance the anti-Mite effects of MF+.

Key words: Perbiotic, feed additive efficacy, reduction of dermanyssidaeinfestation, laying poultry farms alborz

Comparative study of pathological lesions in bovine aborted fetal tissue in neospora caninum cases diagnosed by PCR

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Introduction: Neospora caninum is one of microscopic protozoa that is not known as a factor of aborted fetus in cow, more than goat and sheep and it is a disease in other domestic and wild animals. Parasite infects fetus by way of placenta that this infection can cause aborted fetus at 3-8 months of pregnancy of born calves with congenital infection. For the reason that protozoa are an important factor of aborted fetus all over cattle of cows it causes economic loss to Iran husbandry industry. So histopathologic loss in different members of aborted fetuses can assist us in knowing parasite and combating with it.

Methods: In this way 164 numbers of aborted fetuses were gathered from Alborz in 1 year (1391-1392) and covered with ice, taken into laboratory. After splitting skull, a piece of fetus's brain was separated and kept at temperature of 70 °C and so, is prepared for PCR test.

Results: 21 percent of numbers of aborted fetus's heart, brain, liver, thymus, spleen and pulmonary were positive in their PCR test that is prepared sample of them and are prepared for studying histopathology.

Conclusion: Acquired results show that from 164 number of fetuses, 67 numbers of them were positive after brain PCR test. Therefore 40.85 percent of them were infected with Neospora's parasite.

Key words: neospora caninum, pathological lesions, bovine, abortion, PCR.

Comparative evaluation of two histopathology and PCR methods in detection of toxoplasmosis aborted fetuses in Alborz sheep

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Introduction: Infection caused by toxoplasma is one of the common infections of humans and other warm blended animal. Cats specially ordinary cat play a role as final host and during the wide of animals including birds, ruminant, equines, the first man. especially as the interface host are raised coccidian protozoa. Infection in sufficient per with safety performance, often without clinical protests mu must common manifestation of toxoplasmosis in people will cervical lymphadenopathy is limited to the benign curse. But toxoplasma is able to cause blindness and mental retardation in children infected in the web. As well as sheep, goats, pigs, abortion and infant mortality, causes great economic loses.

Methods: In parasite detection serological and parasitological methods are used. In this study. pathological and PCR method was used in the diagnosis.

Results: in this study, 30 sheep aborted fetuses of a lock that 1 to 5 hours had passed since their deaths. After necropsy a part of the brain in 10% formalin for pathological and another part was frozen al 20C until used for PCR .

Conclusion: After extraction DNA with the help of two GRA6-R GRA6-Fix primers that was made the band equivalent 546bp and PCR done and also frem

Key words: toxoplasmosis, histopathology, PCR, sheep, alborz.

Effects of combination usage Dextran and a new feed additive on CFR at laying Farms Involved by Dermanyssidae mite

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Introduction & Objective: Mites Family Dermanyssidae the most important economic problem is the laying of poultry farms be. Mites with painful bruises and blood pressure can cause stress and decrease seriously egg production. The occurrence of drug resistance against toxins and the problem of chemical pesticide residues has led to Recent studies have focused on the consumption of various herbs, especially their extracts in water Consumption or powder of plants in a Consuming. The purpose of this study Effects of combination usage Dextran and a new feed additive on CFR at laying Farms Involved by Dermanyssidae mite.

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Result: According to the results, the consumption of 1 kg / ton MF+ supplement alone could reduce the population of mites from 62.2 to 12.7. This decrease in Mite population was higher in combination with MF + the Mito (dextran) periotic complement, showing from 64.6 to 10.2. But consuming the Mito supplement alone (dextran) did not show a significant reduction. Effect level MF + supplemented therapy with a single dose of 1 kg /ton alone was 79.58% However, the combined effect of MF + with dextran was 84.21%.

Discussion and conclusion: the therapeutic effect of MF + alone was almost acceptable but because The amount of initial contamination of the farm was very high, this value can be very well evaluated. Using supplements such as Mito can enhance the anti-Mite effects of MF+.

Key words: Perbiotic, feed additive efficacy, reduction of dermanyssidae infestation, laying poultry farms alborz

Analysis of B1 and ROP8 gene regions for designing High-resolution melting technique and separating different types of *Toxoplasma gondii*

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Differentiation and characterization of *Toxoplasma gondii* types plays an important role in the health management and epidemiology of toxoplasmosis. Correct and accurate recognition of the various genetic regions of the parasite is important to select an appropriate region for designing a High-Resolution Melting analysis (HRM). Designing and optimizing HRM analysis for isolating of different types of *Toxoplasma gondii* were main objective of this research. A total of 96 DNA samples of muscle tissue of livestock (cattle, goats and sheep) and poultry brain with three standard strains of *Toxoplasma gondii* (RH, PRU and VEG strains) were prepared and analyzed. Two gene regions of *B1* and *ROP8* were targeted. Nested-qPCR-HRM specific new primers were designed and synthesized. Bioinformatics analyzes were employed to predict the temperature resolution of DNA between different types. To optimize the reaction, 30 positive samples of different types were sequenced and compared with HRM results.

The results of amplified gene regions with innovated primers showed that *B1* gene were able to separate type 1 ($T_m = 84.8^\circ\text{C}$) from two other types ($T_m = 84.6^\circ\text{C}$). Also, the *ROP8* gene was able to separate type 2 with the average melting temperature of 84.5°C from 1 and 3 types ($T_m = 84.12^\circ\text{C}$).

According to the results, *in silico* predictions were completely consistent with the results of the temperature melting behavior of DNA. Also, comparing of temperature variations of two genes with the results of PCR-Sequencing method shows that the *ROP8* gene was better validated than *B1* gene for optimizing HRM reaction.

Histopathologic evaluation of *Allium sativum* methanolic extracts on Liver lesions due to *Echinococcus granulosus*

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Introduction: Hydatidosis, is one of the most important zoonotic parasitic diseases, which caused by the larval stage of *Echinococcus granulosus*. In this study the histopathologic effect of *Allium sativum*(AS) or Garlic methanolic extract on liver tissue of laboratory mice is investigated.

Materials and methods: Seventy laboratory BALB/c mice were infected intraperitoneally by injection of 1.500 viable protoscolices. Five month after infection, the mice were divided into seven treatment groups, including: Albendazol(100 mg/kg), AS methanolic extract (ME)(10ml/liter), ASME (20 ml/liter), ASME (40 ml/liter), ASME (80 ml/liter), Albendazol(50mg/kg) with ASME (10 ml/liter) and untreated control group. The mice received the AS methanolic extract and /or albendazol orally via their drinking water. To evaluate the results of treatment, all mice were euthanized and necropsied one months after the start of treatment. Tissue samples were prepared and studied with light microscopy.

Results: Histopathological changes including hyperemia & congestion, hepatocytes necrosis, hemorrhage and inflammation were compared between the mice of different groups.

The results showed that the lesions decreased with AS methanolic extract. Minimal damages were seen in the group treated with AS methanolic extract at dose of 40mg/liter. However high dose of AS methanolic extract (80mg/liter) led to more tissue damage in liver in comparison of lower doses.

Conclusion: Garlic Methanolic extract caused mild histopathological lesions and decreased tissue damages due to *Echinococcus granulosus*, so can be used as an alternative for albendazol for treatment of hydatid cyst.

Key words: garlic, hydatid cyst, liver, histopathology

Seroprevalence and Associated Risk Factors of *Toxoplasma Gondii* Infection in Schizophrenia Patients in Isfahan, Iran

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introduction: Schizophrenia is widely believed to have a neurobiological basis. Some researchers have found an association between some cases of schizophrenia and toxoplasmosis. The current study was carried out in the aim of to investigate a possible association between *T. gondii* and schizophrenia.

In this study we examined the prevalence of antibodies to *T. gondii* in individuals with schizophrenia and in a matched group of control subjects.

Materials and methods: one hundred and seventy (170) schizophrenia patients and 170 non psychiatric controls were examined for the presence of anti-*Toxoplasma* IgG and IgM antibodies by Enzyme Linked Immunosorbent Assay (ELISA). Then, PCR was performed on samples with positive result in anti-*Toxoplasma* IgM antibody.

Results: From the results, the seroprevalence of anti-*T. gondii* IgG antibodies was significantly higher (31.2%) in schizophrenia patients than in controls (19.4%). There was significant association between the Place of residency and education levels and toxoplasmosis. Anti-*Toxoplasma* IgM antibody was positive in 3 schizophrenia patients and no individuals were positive for this antibody in the control group.

Discussion: Our study is the first to explain the association between *T. gondii* infection and schizophrenia patients in Isfahan, Iran. Our results confirm recent findings that *Toxoplasma gondii* infection is significantly associated with schizophrenia. A positive relationship between Toxoplasmosis and schizophrenia may led to new approaches for the treatment of these disease. This study advanced further weight on the hypothesis that *T. gondii* is a risk factor for schizophrenia. The results also show that *T. gondii* is significantly associated with beef consumption, pica, and risky cat contact.

Key words: *Toxoplasma gondii*, Seroprevalence, Schizophrenia