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On the Cover
Pancreatic tissue in developing ostrich embryo. In this semi-thin and Toluidine blue stained section, the zymogen granules and mitotic cells are evident. Photo: M. Ahadian and Z. Saadatfar. See page 28.
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Annexin A1, A2 and cytokine levels during experimental sepsis in calves

Mohammad Reza Mokhber Dezfouli, Zohre Eftekhari, Masoomeh Heidari Sureshjani,
Mohammad Mahdi Dehghan

Keywords
annexin, calves, cytokines, E. coli

Abstract
Annexins are fundamentally related proteins that process a variety of physiologic and pathologic procedures, including suppression of inflammation. Ten Holstein-Frisian bull calves (10 ± 1 days old) weighting 50 ± 5 kg were chosen to induce the experimental septicemia using O111:H8 strain of E. coli. Blood samples were collected to determine the plasma annexin A1, annexin A2, TNF-α, IFN-γ, IL-8 and neutrophil count at 0, 24, 48, 72, 96 and 120 hours after induction of septicemia. Significant increased concentrations of serum annexin A1 and annexin A2 in circulating blood in response to experimental colisepticemia were observed during experiment. Maximum levels of annexin A1 and A2 were recorded at 72h after challenge. A statistically significant increase in blood neutrophil count occurred from beginning of septicemia until 24h after onset of septicemia. Annexin A2 and Annexin A1 had no significant correlation with neutrophil count. Serum cytokine concentrations reached their maximum level at 48h after challenge and then decreased to basal level before antibiotic therapy. This study showed that serum annexin concentrations, increasing during colisepticemia in calves, in association with cytokines could be a reliable marker to confirm the occurrence of anti-inflammatory response.

Abbreviations
CFU: Colony-Forming Unit
TNF-α: Tumor Necrosis Factor alpha
IFN: Interferon
IL-8: Interleukin 8
ELISA: Enzyme-Linked ImmunoSorbent Assay
Introduction

The inflammatory reaction is a defensive process whereby the body is able to neutralize infections [1]. In the site of the systemic or local inflammation, polymorph nuclear leukocytes, lymphocytes, monocytes and endothelial cells activation lead to release of pro-inflammatory cytokines. Bacterial lipopolysaccharides activate the macrophages and cause release of TNF-α and IL-8 which can have an effect on heart, liver and other organs [2].

The pro-inflammatory phase is capable of inducing several endogenous anti-inflammatory mechanisms that lead to the resolution of inflammation phase. In response to injury, inflammatory cells such as neutrophil granulocytes secrete a number of cytokines into the bloodstream such as IL-1, IL-6, IL-8, and TNF-α [3,4]. Tumor necrosis factor α is a cytokine that has been associated with neutrophil extravasation and enrolment to inflammatory sites [5].

Annexins (also known as lipocortins) are a family of fundamentally related proteins which are classified by way of their ability to bind membrane phospholipids in a Ca^{2+}-dependent mode. One of the main roles of annexins is the regulation of a variety of physiologic and pathologic processes such as suppression of inflammation [3,4]. They are predominantly abundant in a number of cells of the host immune system. Annexin A1 can be transferred from cytoplasm to membrane after activation or after adherence to endothelial cells and be released from neutrophils [6,7,8].

In normal conditions, cytoplasm of immune cells such as neutrophils, monocytes, and macrophages contains high levels of annexin A1. During inflammatory responses in calves, changes in neutrophils occur to a greater extent as compared to other cells. Following cell activation, neutrophils bind to endothelial-cell monolayers and annexin A1 is mobilized to the cell surface and secreted [8, 9]. Annexin A1 promotes neutrophil apoptosis and the apoptotic neutrophils are phagocytized by macrophages [8]. Annexin A1 is also released by apoptotic neutrophils during the process of inflammation and enhances the clearance of apoptotic cells by tissue macrophages that are able to mediate a rapid anti-inflammatory effect [8, 10].

Annexin A2 is involved in various biological functions such as fibrinolysis, angiogenesis, and cell migration, but the exact mechanism of its activity is not understood. In inflammatory dendritic cells, Annexin A2 maintains immunomodulatory activation of cytokines secretion, indicating an important role in normal situation and inflammatory diseases [10, 11].

Although extensive research has been carried out on cytokines and annexins during sepsis, there has been no reliable evidence that shows any correlation between these factors and time span of sepsis. On this basis, we conducted a study to determine the levels of annexins A1 and A2, IL-8, TNF-α, and IFN-γ in the peripheral blood of septic calves over a time span, and to investigate an association between serum Annexins A1 and A2 levels, blood neutrophil count, and serum cytokine levels.

Results

Evaluation of the results of the present study demonstrated that annexin A1 and annexin A2 were elevated in peripheral blood in response to experimental coliseptisemia and these changes over time were significant (p < 0.05). Maximum level of annexin A1 was recorded at 72h after challenge, and the serum levels of annexin A1 at 24, 48, and 72 h (P = 0.039, P = 0.04 and P = 0.045, respectively) were significantly higher than its level in 0 hour (Figure 1).

The serum levels of annexin A2 at 72 hours after colisepticemia increased significantly (P = 0.017) (Figure 2). Repeated measure ANOVA revealed significant changes in serum level of Annexin A2 over time after challenge (p < 0.05).

TNF-α, IL-8 and IFN-γ concentrations in this experiment reached to a maximum level at 48h after challenge. The serum concentrations of TNF-α, IL-8 and IFN-γ were significantly different during the experimental time points (Figures 3 and 4).

The neutrophil count showed significant changes during the study (P = 0.004) and its increase was statistically significant at 24 h after challenge as compared with its level at the beginning of septicemia (P = 0.003) (Figure 5). The white blood cell count decreased due to septicemia (P = 0.0001) (Figure 6).

Discussion

During the inflammation, bacterial immunogenic components stimulate production of pro-inflammatory and inflammatory factors and modify
blood cell numbers and amount of cytokines [2,8]. Neutrophils are critical components of the innate immune reaction and are the preliminary responders to infection. Twenty four hours after bacterial infection they increase in peripheral blood to limit the inflammation.

Activation of polymorphonuclear cells has an important role in sepsis including the release of pro-inflammatory cytokines such as TNF-α and IL-8, that in turn induce immune cell recruitment to the inflammation site [8]. The results of the present study showed that an elevated level of annexin A1 and annexin A2 in the peripheral blood of septic cases may play a part in an active anti-inflammatory function which subsequently contributes to the resolution of sepsis. The enhanced level of annexin A1 during experimental colisepticemia in calves in this study, with the highest recorded level in hour 72, is in agreement with the previous reports [6, 8]. Annexin A1 is an anti-inflammatory protein that plays a key role in innate immunity and modulates the activation of several types of cells such as neutrophils.

We found that in response to infection, the level of annexin A1 and A2 was increased in the peripheral blood after activation and proliferation of neutrophiles. The level of pro-inflammatory cytokines was significantly elevated in all cases with sepsis; and those levels correlated with the levels of AnnexinA1 and A2 in some time points [12].

Body’s defense system suppresses mechanisms through the anti-inflammatory mediators

![Figure 1](image1)

**Figure 1** Variation of Annexin A1 level during experimental septicemia with *E.coli* (Mean ± SE).

![Figure 2](image2)

**Figure 2** Variation of Annexin A2 level during experimental septicemia with *E.coli* (Mean ± SE).

![Figure 3](image3)

**Figure 3** Variation of IL-8 level during experimental septicemia with *E.coli* (Mean ± SE).
such as TNF-α and IL-6 to resolve sepsis. Based on previous studies, serum cytokine disturbance patterns play a key prognostic role in septic shock cases and based on present results, serum annexin A1 reached its maximum level after 72 h compared to other cytokines [6].

In the present study, cytokines reached their peak level at 48 h after challenge and decreased to basal level following antibiotic therapy. Annexin-A1 level had a mild increase after bacterial inoculation and treatment had no effect on its level. This delay in the enhancement of annexin levels may be due to the activation of neutrophiles that subsequently release annexins in blood circulation. On the other hand, annexin A1 has been shown to attenuate leukocyte recruitment in many experimental inflammatory models by inhibiting cell adhesion and migration [1, 18].

Based on Perretti and Gavins findings in 2003, cytokines such as tumor necrosis factor, interleukin-1, and interleukin-8 can also increase cellular and tissue annexin A1 expression [7]. It is clear that when neutrophils adhere to the endothelium and the amount of neutrophiles decrease in the circulation, annexin A1 is released from the neutrophil cytoplasm to the cell surface and thereupon the level of annexin is enhanced [7,14]. In fact, annexin A1 leads to the detachment of adherent leu-
kocytes and indicates that inactivation of adherent cells may be controlled [7].

Based on previous studies, elevation of anti-inflammatory cytokines such as IL-10 and TNF-R1, due to sepsis can result into an enhanced risk of death. An elevation in concentration of annexins A1 and A2 after high level of cytokines found in this study can be an indication of the protective effects of annexins during sepsis[2,12]. However, it is unclear why the annexin A1 levels in the sepsis patients were not correlated in response to neutrophil counts. In previous reports in contrast to present results, annexin A1 levels in sepsis patients were not elevated in response to septicemia reaction. Further studies have to be done to investigate the role of circulating Annexin A1 & A2 in clinical applications among patients with sepsis[8].

Notwithstanding, there was no observed significant correlation between neutrophils count and annexin A changes but these findings suggest that increasing the annexins during the colisepticemia in calves after treatment can be a reliable marker with regard to cytokines, confirming the occurrence of anti-inflammatory response after activation of neutrophiles. It is hypothesized that the annexin proteins have anti inflammatory roles in the inflammation phase by decreasing the infectious cells and enhancing the immune defense factors. It is still a controversial issue as to whether or not the circulating level of anti-inflammatory mediators can be used as a prognostic factor to predict case survival.

Materials and Methods

Animals and experimental preparations

Ten Holstein-Frisian bull calves 10 ± 1 days of age with body weight of 50 ± 5 kg were selected for study. The calves were fed colostrum [10% BW] within six hours of birth. They were housed in individual stainless steel pens [1 m × 1.5 m × 1m] with a chaff coated floor and were fed twice daily with whole milk at the rate of 10% of their body weight per day divided into 2 feedings at 7:30 and 16:30 [15]. Water and starter provided ad libitum. The calves’ vital signs (temperature, heart and respiratory rate) were checked before experiment [12,14].

The O111:H8 strain of *Escherichia coli* was chosen for inducing colisepticemia. This strain is commonly used in experimental studies since it is rapidly phagocytized, and produces a robust oxidative burst [16,17]. To induce experimental septicemia, a catheter was inserted in the jugular vein and an *E. coli* suspension [1.5 × 10⁹ CFU] in 5 ml isotonic saline was inoculated as a bolus.

Biochemical and hematological analysis

After observation of the septisemia symptoms based on accepted criteria including altered appetite and behavior, shock signs, standing ability, and suckling reflex and hematology confirmation, blood samples were collected into 6-mL tubes containing EDTA for determination of plasma annexin A1, annexine A2, TNF-α, IFN-gamma, IL-8 and blood cell count at 0 [inoculation time], 24, 48, 72, 96 and 120 hours after challenge. Four ml of peripheral blood was collected into a sterile syringe after monitoring the calves and observing the septic shock symptoms and injected to a Diphasic media and incubated at 37°C for 24 h to confirm septicemia in calves. Then pure culture and serotyping was performed to detect the isolated bacteria and to confirm the *E. coli* strain O111:H8. The measurement of serum levels of annexin A1 and annexin A2 were carried out by ELISA (Casabio, Australia). The serum levels of TNF-α, IFN γ and IL-8 were determined by related ELISA kits (AbD Serotec®, A Bio-Rad Company, Kidlington, Uk).

For ethical reasons, all calves were treated with a suitable antibiotic which had been selected by antibiogram. Treatment with antibiotic started 36h after bacterial administration with Cefazidime (ZACZIDIM®, DAANA Pharma Company, Tehran, Iran) at a dose of 10 mg/kg IV TID for 3 consecutive days.

Statistical Analysis

The data were analyzed with repeated measures ANOVA using SPSS version 13.0 software and significance level was considered as p less than 0.05. The correlation between annexin A1 and A2 and cytokines concentration and neutrophile counts was studied using Pearson’s correlation coefficient.

Acknowledgements

The authors gratefully acknowledge the financial support provided by the Iran National Science Foundation [INSF] and Institute of Biomedical Research of Veterinary Medicine, Tehran University.

Author Contributions

Desinged the study and conducted the systematic literature review: Z.E., M.R.M.D. and M.M.D. Analyzed the data and drafted the manuscript: Z.E. Conducted the experiments and participated in in-vivo studies: Z.E. M.R.M.D. and M.H.S.

Conflict of Interest

The authors declare that they have no competing interests.

References


Serological and molecular studies on visceral leishmaniosis in stray dogs in Torbat-e-Heidareih area

Mohsen Pourali, Gholamreza Razmi, Sadaf Sabzavari

Keywords

Leishmania infantum, stray dogs, Torbat-e-Heidareih area

Abstract

Visceral leishmaniosis is an important zoonotic disease in Iran. The agent, vector and reservoir are Leishmania infantum, Phlebotomous major and dogs, respectively. Thus far, no data has been reported about the prevalence of visceral leishmaniosis in human and dog in Torbat-e-Heidareh area and the present study has been performed to determine the frequency of L. infantum infection in stray dogs. One hundred blood and skin samples were collected from 2014 to 2015. The sera samples were examined by Indirect Immunofluorescent Antibody Test (IFAT). The skin samples of seropositive and seronegative in association with skin lesions were examined by PCR method. In this study, antibodies against L. infantum were detected in 3 (3%) sera samples of dogs and kDNA Leishmania spp. was detected in 4 (4%) of seronegative dogs by PCR. L. infantum was detected in positive samples by semi nested PCR. Based on serological and molecular results, 7% of asymptomatic stray dogs of Torbat-e-Heidareh area were infected with L. infantum and may be acted as reservoirs of visceral leishmaniosis.

Abbreviations

IFAT: Indirect Immunofluorescent Antibody Test
kDNA: Kinetoplast DNA
L. infantum: Leishmania infantum
Introduction

Visceral leishmaniosis is known as a zoonotic disease. Dogs are reservoirs of infection for people [1, 2]. *Leishmania infantum* and *Leishmania chagasi* are the main causes of zoonotic visceral leishmaniosis, while the anthropotic infection is caused by *L. donovani* in India and East Africa. In the Middle East, zoonotic visceral leishmaniosis is caused by *L. infantum* with domestic dogs as its major reservoir host [1, 2]. The prevalence rates of canine leishmaniosis are different in endemic areas and depend on the climatic conditions. The prevalence rate of disease was reported between 10 to 70% in the Mediterranean area. [3, 4]. So far, visceral leishmaniosis have been reported in dogs and wild carnivores as the main reservoirs from different parts of Iran [5-8]. Canine leishmaniosis is a chronic disease and characterized clinically by lymphadenopathy, cutaneous signs, weight loss and anemia. In addition, many infected dogs are asymptomatic and serve as reservoir hosts of disease in endemic areas [9-11].

The molecular methods have high sensitivity and specificity for the demonstration of every pathogen in human and animal. The PCR assay has been significantly improved the diagnosis of visceral leishmaniosis in man and animal [12]. The range of frequency of visceral leishmaniosis has been determined to be 7-8.5% in dogs of Mashhad area [13-15]. Due to the proximity and similarity of climatic condition between Mashhad and Torbat-e-Heidareih and lack of data about visceral leishmaniosis in this area, the objective of this study was to determine the prevalence of zoonotic visceral leishmaniosis in asymptomatic stray dogs in Torbat-e-Heidareih area by IFAT and PCR methods.

Results

A total of 100 sera of 85 male and 15 female asymptomatic stray dogs were examined by IFAT. In this study, seroprevalence of *L. infantum* infection was determined %3 (3/100) in asymptomatic stray dogs. The kDNA of *L. infantum* was detected in 4 (4/100) of the skin samples in seronegative stray dogs (Table 1) (Figures 1 and 2). All positive samples belonged to male stray dogs, but the frequency rate in male and female dogs was not significantly different ($p < 0.05$) There was not any agreement between IFAT and PCR methods. (Kappa= -0.036)

Discussion

In the present study, the antibodies against *L.
infantum was detected in 3 serum samples (3%) of asymptomatic stray dogs. This rate was lower than the seroprevalence of canine leishmaniosis that has been reported in Mashhad area [13-15]. The seroprevalence range of canine leishmaniosis has been determined from 4.4% to 18.2% in other provinces of Iran [11, 16-18]. Difference in the prevalence of canine leishmaniosis in above studies may be due to the difference in climate of sampling areas and various serologic methods.

In the present study, Leishmania DNA was detected in 4% of skin samples in asymptomatic seronegative dogs by a genus-specific PCR [19]. This finding is in agreement with other studies in the world [20-24]. Two reasons may be caused this results: 1) the seronegative/PCR positive dogs belong to the resistance group that is characterized by strong cell-mediated immunity with low antibody production, 2) the dogs may have been recently infected and the antibodies titer is non-detectable against L. infantum.

Our study showed that the frequency of infection in male and female dogs was not significant. Similar results have been reported by other researchers in Iran [8, 11, 15, 16, 17], although some studies showed that the frequency of visceral leishmaniosis in males is higher than female dogs. [22-24].

In this study, there was not any agreement between PCR and IFAT methods. Previous studies have also reported a poor agreement between PCR and serology methods [25-26]. The leishmanial species of all PCR –positive sample were identified as L. infantum by a semi nested PCR [27]. This results supports the results of many epidemiological studies that L. infantum is the main causative agent of canine leishmaniosis in Iran [7, 22]. Based on the serological and molecular examination, it seems that asymptomatic stray dogs could act as reservoirs of visceral leishmaniosis in this area. Further epidemiological studies are recommended to determine the prevalence of visceral leishmaniosis in human population in this area.

Materials and Methods

Study area

The study was performed from February 2013 to February 2014 in Torbat-e-Heidreh that is located at 35.17˚ north latitude and 59.12˚ east longitude, in Khorasan Razavi Province, Iran. The average annual temperatures and precipitation are 21 °C and 250 mm, respectively. Summers are typically hot and dry, with high temperatures sometimes exceeding 35°C (95 °F). Winters are typically cool to cold and somewhat damper, with overnight lows routinely dropping below freezing.

Sample size

The prevalence of leishmania infection was estimated 7% in stray dogs of Mashhad area. Based on this prevalence, the desired sample size was 100 stray dogs, using a 95% level confidence and 5% desired absolute precision (28). One hundred asymptomatic stray dogs were randomly selected during a zoonotic disease control program that was done by Torbat-e-Heidareh Municipality. The blood and skin samples were collected. The blood tubes were transferred to laboratory in cool condition. The clotted blood was centrifuged at 800 ×g for 5-10 min. The sera and skin samples were stored at -20˚C before serological and molecular examination.

The indirect immunofluorescent antibody test (IFAT)

In the present study, IFAT was used as sero-diagnostic tool. The L. infantum antigen (Megacor Co, Austria MegaScreen FLUOLEISH) has been used for this study. For each reaction, 10 μl of serum dilution (1:50) in PBS was added over the slide holes. The slides were incubated in a hummid chamber at 37°C for 30 min. The slides were washed for 5 min in PBS. The conjugate was diluted in 1:200 in PBS with 0.025% Evan’s Blue and then 15 μl of this solution were placed over the slide holes. The incubation and washing steps were repeated once, as outlined above. Slides were mounted with buffered glycerin, covered with a cover slip and read under an Olympus BX-FLA fluorescent microscope equipped with a 100W mercury lamp with 400× magnifying power. The diluted serum showing an evident yellow-green fluorescent signal upon microscopic examination was accepted as positive IFAT titer.

DNA extraction

Genomic DNA of skin samples of seropositive dogs and
the seronegative dogs were extracted using MBST Genomic DNA kit (Institute of Molecular and Biological transmission systems, Tehran, Iran) as per manufacturer’s recommendations. The extracted DNA was stored at -20°C for examination of *Leishmania* kDNA detection.

**PCR assay**

*Leishmania* genus-specific oligonucleotide primers K13A (5′-dGTGG GGGAGGGGCGTTCT-3′) and K13B (5′-dATTTTACACACACCCCCAGTT-3′) described by Rodgers et al. (19) were used to amplify a 120 bp fragment in the conserved region of *Leishmania* kDNA mini-circles.

To identify *Leishmania* species, an assay based on the semi nested PCR was used for amplification of variable area of the mini-circle kDNA (with a slight modification) as described by Aransay, et al. [27]. Negative (H2O) and positive controls were used for each experiment. DNA of positive control was prepared from *Leishmania* culture [29]. After amplification, the PCR products were electrophoresed in 2% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light.

**Statistical analysis**

The Seroprevalence rate of *L. infantum* in male and female dogs were statistically analyzed using Chi square. The agreement between IFAT and Semi nested PCR were assessed by Cohen's kappa test. The agreement between the different tests is shown as k value. The agreement is poor if k values are between 0.2 and 0.4, moderate if k values are between 0.4 and 0.6, substantial if 0.6 and 0.8, and good if it exceeds 0.8 [30].

**Ethical consideration**

Study protocols and methodologies were revised and approved by the Ethical committee of Ferdowsi University of Mashhad.

**Acknowledgements**

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**Author Contributions**

Collected samples: M.P. Performed IFAT and PCR: M.P. and S.S. Designed the study, and set up IFAT and PCR, wrote, and edited the manuscript: G.R.

**Conflict of Interest**

The authors declare that they have no competing interests.

**References**


Visceral leishmaniosis in stray dogs


The investigation on the relationship between dairy cow hygiene scores and intramammary infections

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Keywords
hygiene scores, intramammary infections, dairy cow, California mastitis test, somatic cell count

Abstract
The “cow hygiene score” system is a management tool for assessment of cow’s body hygiene and cleaning of the barn. In this study, the correlation between body hygiene scores and intramammary infections (IMI), California Mastitis Test (CMT) and Somatic Cell Count (SCC) were evaluated in two different seasons. Hygiene scoring of 1096 dairy cows in 4 herds was performed on five body areas including: udder, rear legs, flanks and upper legs, abdomen, and tail head. After doing CMT, milk samples were taken from quarters with score 2 or more for microbiological culture. SCC data were taken in two consecutive months. The results of this study showed no significant differences between the median of all hygiene scores except for tail head that were significantly greater in high than low rainy seasons (p < 0.05). There was no significant correlation between hygiene scores and the chance of positive bacterial culture (p > 0.05), but a statistically significant relationship was found between udder hygiene score and isolation of environmental bacteria (p < 0.05). There were no statistically significant differences between SCC or CMT and hygiene scores of all parts of the body and similarly between teat cleanliness score after premilking preparation and SCC in two consecutive months (p > 0.05). Finally, it seems that udder hygiene scoring is an useful tool for predicting of intramammary infections caused by environmental bacteria.

Abbreviations
SCC: Somatic Cell Count
IMI: Intramammary Infections
CMT: California Mastitis Test

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Introduction

Mastitis in subclinical or clinical forms can result in significant reductions in animal welfare and productivity of the dairy herd. The organisms that cause mastitis can be classified as “contagious” or “environmental. Contagious infections occur when teats of healthy cows are exposed to bacteria present in milk (coming from infected udders), and environmental mastitis is caused by bacteria found in housing areas. Moisture, mud, and manure are common sources of these pathogens. Exposure to manure in housing areas and poor hygiene in cows can influence the rate of clinical mastitis [8]. Successful control of environmental pathogens is based on efficient pre-milking preparation and cleanliness of farm environment. Bartlett et al. [1992] showed that an index of environmental sanitation based on the amount of manure on the cow was able to predict the occurrence of clinical coliform mastitis [3]. The use of subjective measures like body condition scoring, lameness scoring, and teat condition scoring are effective means of assessing animal health and herd management. The cow hygiene score system is a management tool to evaluate and monitor cow cleanliness and the farm environment [1-3,5,6]. The cow hygiene scorecard is broken down into five general areas: Tail head, Flank, Belly, Udder, and Rear legs and feet. These scores use simple drawings to illustrate the degree of cow hygiene. Furthermore teat end cleanliness is a good indicator of the effectiveness of pre-milking cow preparation. Most studies that have evaluated the hygiene of dairy cows had been performed with animals housed in free-stalls. In addition, little information is available about relationship between type of micro-organisms and season variation of these scores.

Results

In this study, we did not find any significant differences between the median of all hygiene scores except for tail head that were significantly greater in high than low rainy seasons ($p < 0.05$) (Table 2). In overall, 461 milk samples were taken from quarters with score 2 or more in CMT. After microbiological culture, 191 microorganisms were isolated from milk samples of 108 cows. Major bacterial isolates were contagious bacteria including *Staphylococcus aureus* (39.7%), *Streptococcus agalactiae* (7.85%), *Corynebacterium* (7.32%) and environmental bacteria included coliforms (8.9%), *bacillus cereus* (3.14%), environmental streptococcus (13.61%) and yeast (1.57%). Other isolated agents were negative coagulase *staphylococcus* (17.8%). Statistically, there was no significant correlation between hygiene scores and the chance of being positive in bacterial culture ($p > 0.05$). Udder hy-

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<td>Data collected from <a href="http://www.wunderground.com">www.wunderground.com</a></td>
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Therefore, the aim of this study was to investigate if there is any correlation between hygiene scoring and measures of IMI by isolation of bacteria, CMT and SCC in cows that are not housed in stalls and to determine the effect of season variation on hygiene scores.

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<td><strong>The relationship between hygiene scores in different seasons</strong></td>
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<td><strong>Median</strong></td>
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<td><strong>Upper quartile</strong></td>
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Values within columns with different superscripts (a, b) are significantly different ($p < 0.05$).
giene scores showed a significant relationship with isolation of environmental bacteria of the same cows \((p < 0.05)\), but there was not any significant difference between other hygiene scores and isolation of environmental bacteria \((p > 0.05)\) (Table 4). The probability of isolation of environmental bacteria in cows with hygiene score of 4 was greater than cows with score 1 (12.8% vs. 0%) \((p < 0.05)\). There was no significant statistical differences between SCC or CMT with hygiene scores of all parts of the body \((p > 0.05)\). There was not any difference between teat cleanliness score after pre-milking preparation and SCC in two consecutive months \((p > 0.05)\).

**Discussion**

The rate of infection is decreased by utilization of proper milking hygiene, properly functioning milking machines, and teat dipping, while the duration of infection is controlled by treatment of infections and culling. The purpose of mastitis control programs are prevention of new infections and elimination of existing infections. Poor cow hygiene can contribute to presence of mastitis pathogens on teat ends and increasing the rate of new infections. Our results did not find significant differences between the median of all hygiene scores except for tail head that were significantly greater in high than low rainy seasons. Our results do not support the previous study [7] that observed the dirtiest cows in summer (January – March) and related it to the raining and increasing of humidity in this season and its negative effect on cows’ hygiene scores. According to Table 1 there is not a large difference in the amount of rainfall in most cities of Iran including Mashhad. Thus, the hygienic scores do not have a seasonal pattern in the studied area. In present study, the significant and inverse relationship between tail head hygiene score and season can be attributed to using of mist cooling in low rainy season in these farms that increasing the moisture levels in the tail head. This may cause more manure to adhere to this area of the body.

In the present study absence of a significant
relationship between hygiene scores of all parts of the body with bacterial isolation can be attributed to dominant pathogen of mastitis is in studied farms. In our study, more than half of the isolated bacteria were contagious pathogens. In one hand, the transmission pattern of this pathogens are different from the causative agents of environmental mastitis and less dependent on the hygiene score of the cows and on the other hand, by entering the causative agent of contagious mastitis to the udder, there will be no opportunity for engagement of environmental pathogens. Thus, it is not unexpected that there was not any correlation between the hygiene scores of the cow and the absolute separation of bacteria. Furthermore, detection of a significant correlation between udder hygiene score and isolation of environmental bacteria in studied cows can be attributed to direct contact of udder and teat with the bed and easier penetration of bacteria into the mammary tissue. In a similar study [8] it was found that the risk of contamination with main environmental pathogens in cows with scores 3 and 4 is approximately 1.5 times of cows with scores 1 and 2. Although previous studies have found that by increasing the hygiene score of the body, levels of SCC are increased [4,6,8,9], in this study, absence of significant relationship between hygiene score of the body and SCC can be related to the dominant pattern of mastitis pathogens (contagious mastitis). Occurrence of this result is not unexpected because in farms with high prevalence of contagious mastitis, high somatic cell count mostly is related to contagious pathogen rather than exposure to environmental pathogens. In conclusion, it seems that there was not a significant relationship between the hygiene scores and SCC or CMT that are mostly increased in response to contagious mastitis. In addition, udder hygiene score is a useful tool for prediction of intramammary infection which is caused by environmental bacteria.

Materials and methods

The present study was performed on 1096 Holstein dairy cattle in 4 herds in Mashhad, Iran. In dairy farms with populations of total cows below 100, all cows, and in farms with over 100 heads of milking cows, 25% of cows in each barn were included in the study. A scoring system scale from 1 to 4 was selected. The cow hygiene score was carried out on five areas of each animal’s body: udder, rear legs, flanks and upper legs, abdomen, and tail head [6,8]. The scores were defined as follows: 1 = entire area was clean, with no dirt; 2 = 2-10% of the surface area was dirty; 3 = 10-30% of the surface area was covered with dirt; 4 = >30% of the surface area was covered with caked on dirt. Teat Cleanliness Score performed using a 4-point scale to assess the degree of manure and bedding contamination at the teat end after completion of the preparation procedure, prior to unit attachment. The scores were defined as follows: 1=Clean: no manure, dirt, or teat dip solution; 2=teat dip solution is present, no manure or dirt; 3=Small amount of dirt and manure is present; 4=Larger amount of dirt and manure is present; *Within each column values with different superscripts represent significant differences (p < 0.05)
dirt and manure is present. The CMT was done and milk samples were taken from quarters with score 2 or more in CMT and cultured on microbiological medias. Collection of milk samples and microbiological procedures were performed as outlined by the National Mastitis Council [National Mastitis Council, 1999]. SCC data were taken in two times, simultaneous with hygiene scoring and one month later for the present status of the animals and the effect of hygiene on SCC, respectively. It seems that the level of raining and season may affect contamination of cow’s body with manure and therefore hygiene scoring, so this study was performed in two seasons [high and low rainfall]. Average monthly rainfall in two different sampling times for each farm are shown in Table 1. To compare the SCC and hygiene scores, Kruscal-wallis test was used. Spearman correlation test was used for surveying the relationship between hygiene score and CMT. The relationship between hygiene score with the culture results were evaluated by Chi square and Fischer’s exact tests. Comparison between hygiene score in each farm in two seasons [high or low rainy] were analyzed by Mann-Whitney U test.

Acknowledgements

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Author Contributions

Conceived and designed the experiments: B.K., S.A.T.R. Performed the experiments: I.R. Analyzed the data: M.A. Wrote the paper: BK, S.A.T.R.

Conflict of Interest

None of the authors of this paper have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

References


Isolation and molecular diagnosis of Peste des petits ruminants (PPR) virus from contaminated areas in Iran

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Keywords

PPR, isolation, diagnosis, Iran

Abstract

Due to the numerous reports concerning the Peste des petits ruminants (PPR) in different regions of Iran, the isolation and genetic characterization of native isolates is very important. During 2013-2014, 168 samples were collected from whole blood, eye, nasal and oral swabs, and lymph nodes of sheep and goats with clinical signs in nine provinces with infected focal zones in Iran. Vero cell line and primary Lamb Kidney cells were inoculated with isolates and observed at least in 5 consecutive passages for cytopathic effects (CPE). The sheep samples from two provinces, created CPE in both kinds of cell cultures. Observation by electron microscopy and confirmation by RT-PCR was used to confirm PPR.
**Introduction**

Peste des Petits Ruminants (PPR) is an acute and highly contagious viral disease of goats and sheep causing high morbidity and sometimes high mortality rates [1-4]. The disease is characterized by fever, necrotic stomatitis, gastroenteritis, pneumonia, and sometimes death [5]. The causative agent of the disease, PPR virus (PPRV), is classified as a member of the genus Morbillivirus in the family Paramyxoviridae [4, 6].

PPR was first reported in Cote d'Ivoire (the Ivory Coast) in 1942 and subsequently in other parts of west Africa [7, 8]. The disease occurs in a band that spreads across Africa between the equator and the Sahara, through the Arabian Peninsula, the Middle East, south-west Asia and India [9]. In China, the disease was reported in 2007. Following this report, in 2008, the disease was reported in North Africa, Morocco [5, 10]. In the last 15 yr, PPR has been reported in Turkey and Iran [3, 11]. In recent years, there are many reports of diagnosis of PPR in Iran but the causative virus has not been isolated [11]. In this study, a specific cell culture condition and specific primers were used for virus isolation and molecular identification of PPRV from samples of sheep and goats in infected zones.

**Results**

Kits for rapid detection of morbillivirus in samples of eye and nasal swabs in two sheep farm from East Azarbaijan and Kerman provinces identified positive samples (Figure 1). In samples of whole blood and lymph nodes from two farms of East Azarbaijan and Kerman that were positive in the rapid detection method, after 4-5 days of inoculation onto Vero and LK cells, the CPE was observed and completed after 24-48 hours. In samples with cell lesions, the CPE observation were performed within 2-3 days after inoculation and completed within 12-24 hours (Figures 2 and 3).

In samples possessing cell lesions, the viral particles (150-300 nm) were similar to paramyxovirus particles in shape and size (Figure 4). These cells (containing lesions), were diagnosed positive by RT-PCR and nested PCR (Figure 5).

**Discussion**

Figure 1
Test for rapid detection of morbillivirus. Positive (left), negative (right).

Figure 2
CPE observed in Vero cells, 3 days after inoculation of buffy coats of sheep, in third passage (magnification: x100).

Figure 3
CPE observed in LK cells, in the first passage of sheep buffy coats (magnification: 100x).
This study was performed to isolate PPR virus circulating in sheep and goats population in contaminated areas of Iran. In this study, the presence of PPR virus was demonstrated by clinical signs, virus isolation and molecular diagnosis. The RT-PCR is an effective test for diagnosis of PPR from field samples and identification of PPRV in cell culture supernatant. The specific primers based RT-PCR is now accepted as an alternative for virus isolation and diagnosis of PPR by Office Internationale des Epizooties for being simple, rapid, highly specific and sensitive [12, 13].

Initially, primary LK cells were employed for the isolation of PPRV from the field samples to increase the sensitivity of this technique. Later, Vero cells were preferrentially used because of their continuity and having lesser chances of contamination. The Vero cells also proved suitable for the isolation of PPRV. CPEs observed in this study due to PPRV on LK and Vero cells were initial cell rounding, detachment from the surface, retraction, vacuolation and multi-nucleate syncytia formation. Other studies have also reported similar findings [13-16].

The two PPRV isolates obtained on primary LK and Vero cells were confirmed using RT-PCR. This low success rate for the isolation of PPRV may be attributed to the fact that the samples were collected at the time of necropsy after disease had run its full course. Successful isolation of PPRV depends on various factors including the phase of the disease during which samples were collected from donor animals [13, 17]. The samples collected during the infectious period i.e. in febrile phase are ideal for the isolation of PPRV. Following the regression of the fever the titers of infectious virus decline rapidly [13, 14]. The isolation of PPRV using Vero cells is reported to be difficult since it requires one or more blind passages to become tissue culture adapted, even from very fresh clinical samples or isolated virus [13, 14, 18].

The RT-PCR using primers for highly conserved sequences within F gene of PPRV proved suitable for diagnosis and/or confirmation of the PPRV isolates obtained on cell culture and effectively tracked the changes in virulence of PPR virus. The assay described by Forsyth and Barrett, (1995) has extensively been used for the specific diagnosis and molecular epidemiological studies of PPR virus [3, 13, 17]. The results of the present study are in agreement with the report of Bahadar et al. (2009), who reported that detection of PPR in buccal mucosa, nasal and ocular discharges was applicable by virus isolation using Vero cell cultures [19]. Also the present study is closely related to the previous findings by Housawi et al., (2004); Libeau et al., (1995); Forsyth and Barret, (1995) regarding isolation of PPR virus using Vero cell line [20, 21]. We applied the methodology which has been reported before to confirm the outbreaks.
caused by PPRV in India, using Vero cell line for isolation of PPR virus, and amplification of PPRV F gene by RT-PCR [22]. Many researchers recommend the RT-PCR method for confirmation of PPRV [4].

Materials and Methods

Sampling
During 2013-2014, 168 samples were collected from whole blood, eye, nasal and oral swabs, and lymph nodes (bronchial, mediastinal, and mesenteric) of sheep and goats with clinical signs in Ghom, Fars, Ardebil, Ghazvin, East/ West Azarbaijan, Lorestan, Tehran and Kerman provinces. The samples transferred to the virology laboratory under standard conditions (in less than 12-24 hours and on ice) and kept in -70°C until use. In animals with clinical symptoms the samples were taken from nasal and eye discharge. Then the samples were evaluated with rapid morbillivirus detection kit (Svanodip-Sweden).

Sample preparation and inoculation
Following the centrifugation at 117g for a period of 10-15 minutes the buffy coat layer was removed from blood samples. Also, 10% suspension was prepared from the homogenized lymph nodes. The prepared samples (buffy coat and homogenized lymph nodes) were inoculated (50 μl/cm²) on Vero cell line and Lamb Kidney (LK) primary cells. Following an hour of inoculation, DMEM (GIBCO) containing 2% FBS (GIBCO) was added to cell culture. A period of 14 days was considered to investigate each sample for cytopathic effects (CPE). In the absence of CPE, the blind passages were performed after two freeze-thawing steps and this was continued for at least 5 times.

To observe the viral particles using electron microscopy (EM), the affected cells were subjected to two times freeze-thawing procedure, and centrifugation at 6000 and 53000g. Then, the precipitated pellet was removed to be evaluated under a Philips 400 electron microscope.

RNA extraction and RT-PCR
Cell cultures with cell lesions were used for extraction of RNA by RNA extraction solution (Cinnagen) according to manufacturer’s instructions. Reverse transcription (RT) reaction was performed using Revert Aid First Strand cDNA synthesis kit (Fermentas). The RNA and random hexamer primers were heated at 65°C for 5 minute and the thermal procedure was performed as follows: 25°C (5 minutes), 42°C (60 minutes) and 70°C (5 minutes). PCR reactions were performed using 2x PCR Master Mix (Cinnagen). The following primers were used [17, 23]:

PPRV F, 5' – ATCACAgTgTTAAAgCCCTgTAgAgg– 3'
PPRV R, 5' – gAgACTgAgTTgTgACCTACAAgC– 3'
PPRV F(Nested), 5’ – ATgCTCTgTCAgTAgATAACC– 3'
PPRV R(Nested), 5’ – TTATggACAgAAggACAAg– 3'

Thermal program for amplification of PPR F was: 94°C (45 seconds), 60°C (30 seconds) and 72°C (20 seconds) for 22 cycles, starting with an initial denaturation of 94°C (4 minutes) and a final extension of 72°C (5 minutes).

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Author contributions

RF participated in the design of the study, writing the manuscript, final approval of the version to be published and managing the research of Animal Viral Diseases laboratory.

Conflict of Interest

None of the authors of this paper have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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Histomorphometric and ultrasonographic evaluations of the rumen in sheep

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Keywords
ultrasonography, histomorphometry, rumen, sheep

Abstract
Rumen lesion can lower the performance of the animal and sometimes cause its death. Ultrasonography as a diagnostic method for the detection of lesions in the gastrointestinal tract is considered safe. In this study, three regions of rumen including the dorsal blind sac, ventral blind sac, and pillar [0.5 × 0.5 cm] from 10 healthy sheep rumen were sampled. Histomorphometric study of all samples were performed in the mucosal, submucosal, muscular and serosal layers. For ultrasonographic evaluation, samples from wall of rumen in 6 × 6 cm dimensions were used probe. The results showed that identification of all layers of rumen wall is feasible in sheep by histomorphometry and ultrasonography techniques. Statistical analysis of the data showed no significant correlation between the parameters of the rumen wall in ultrasonography and histological study. The lack of correlation between ultrasonography and histological data may be due to the tissue changes which would occur during the process of preparing the tissue samples including tissue fixation, dehydration and clearing.

Abbreviations
L: Lumen
E: Epithelium
Vi: Villi
LP: Lamina properia
ML-SM: Mucosal and submucosal
MT: Muscular Tunica
H&E: Hematoxylin and Eosin

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Movahedi Zade/Raji/Mirshahi
Introduction

Ruminant stomach has developed four separate compartments, each with its own morphologic particularities. The first three parts are rumen, reticulum and omasum; commonly known as forestomach [1]. There has been considerable research into the organization of the stomach in cattle [2], sheep and deer [3], and goat [4, 5]. The rumen is itself sacculated by muscular pillars into what are called the dorsal, ventral, caudodorsal, and caudoventral blind sacs. The rumen has a keratinized stratified squamous epithelium. It is non-glandular and has no lamina muscularis. There are two thick layers of tunica muscularis, the inner circular and the outer longitudinal [6]. The anterior surface of rumen forms numerous papillae. The papilla can be long and foliated and pointed. They are up to 6 mm in length. Animal fed on rough grass or in the dry season have longer papillae, whereas animals fed on digestible feed or in wet season have shorter papillae.

Ultrasonography is a diagnostic imaging technique based on the application of ultrasound. Compared to other prominent methods of medical imaging, ultrasound has several advantages. It provides images in real-time, it is substantially lower in cost, and it does not use harmful ionizing radiation. Ultrasonography has been successfully employed in commercial livestock for the past 30 years to determine fetal number and gestation length, permitting more precise feeding and management during late gestation [7]. Ultrasonography examination can also yield important clinical information of lesion in the chest [8], reticulum [9], forestomach [10], liver [11], bladder and kidney. [12]. Ultrasonography is an ideal diagnostic tool for the investigation of bovine gastrointestinal disorders, the most common of which are traumatic reticuloperitonitis, left and right displacement of the abomasum, ileus of the small intestine, and dilatation and displacement of the cecum [13]. Ultrasonographic examination of digestive system in 21 normal healthy camels provided highly useful information of ultrasonographic appearance of the digestive system which can be as a reference in suspected cases with malformation of the gastrointestinal tract. In this report, the differentiation between the renal cortex and medulla was also clearly visible in the ultrasonograms [18].

Regarding the lack of ultrasonographic data about rumen layers, this study was aimed to associate histomorphometry and ultrasoniography findings in the rumen.

Results

In the histological images of the rumen, the different layers were determined such as, epithelium, lamina properia, tunica mucosa and submucosa, tunica muscularis and serosa (Figues 1, 2, and 3). We concluded that highest average diameter of the mucosa and submucosa was seen in the ventral blind sac of rumen, the highest average diameter of muscle and serous in pillar, and maximum diameter of the walls in pillar (Table 1).

In ultrasonographic images, rumen was seen in the vicinity of the wall of abdomen and its wall is seen as an echogenic thick and smooth line. The structures between its wall and skin body is clearly specified using this technique, however, identification of rumen layers have not been reported [14].

Ultrasonographic examination of abomasum in 50 normal healthy cows showed that ultrasonography is a valuable technique for determining the size, location and content of the abomasum. In most cases, the wall of abomasum was seen as a thin line and also some of its folds were seen like echogenic line structures [15]. Ultrasonographic study of the abomasum in Holstein calves fed before and after the ingestion of milk showed milk clots with clear margin. [16]. The morphological changes in the reticulum were examined by ultrasonography and radiography in 26 cows with traumatic reticuloperitonitis. Radiography revealed foreign bodies penetrating the reticulum of 12 cows and magnets in the reticulum of seven cows. None of these foreign bodies or magnets could be visualized by ultrasonography. Ultrasonographic examination to confirm the diagnosis in animals with unclear and abomasum displacement have also been useful [17].

Ultrasonographic examination of digestive system in 21 normal healthy camels provided highly useful information of ultrasonographic appearance of the digestive system which can be as a reference in suspected cases with malformation of the gastrointestinal tract. In this report, the differentiation between the renal cortex and medulla was also clearly visible in the ultrasonograms [18].

Regarding the lack of ultrasonographic data about rumen layers, this study was aimed to associate histomorphometry and ultrasoniography findings in the rumen.
ameter of the muscular and serous layers was highest in the pillar, dorsal blind sac and ventral blind sac (Table 2).

All measured data were normally distributed; however, there was no significant correlation between the histomorphometric and ultrasonographic data.

**Discussion**

In this study, for the first time it was possible to correspond and match the layers of the rumen wall in ultrasonographic images with histological images. In ultrasonography, the mucosa and submucosal layers appeared more hyperechoic than the muscular layer which was hypoechoic. In ultrasonographic images, distinguishing mucosal layer from submucosal layer was not possible. In this study, for the first time, thickness of the various layers has been reported in the histological and ultrasonography images.

Statistical analysis of the data showed no significant correlation between the parameters of the rumen wall in ultrasonography and histological study. This could be due to changes in tissue parameters during preparation (dehydration, clearing and infiltration).

The average thickness of the rumen wall in histological images was higher in the ventral blind sac, dorsal blind sac and pillar. The average thickness of these layers in ultrasonography was also high. The average thickness of mucosa and submucosal layers in histological images were higher in ventral blind sac, dorsal blind sac and pillar. Also, the average thickness of musculir and serosal layers tissue in histological images were higher in pillar, dorsal blind sac and ventral blind sac.

The most striking result of this study is the determination of all layers of rumen wall in sheep by histological and ultrasonography techniques. It is worth noting that this procedure has been performed for the first time and can be helpful as the first step for future studies and research.

**Materials and Methods**

In this study, 10 rumen from healthy sheep rumen were obtained used for histomorphometric and ultrasonographic examination. For histomorphometric evaluation of the rumen, the samples were taken from the three areas of the rumen wall including the dorsal blind sac, ventral blind sac, and pillar (0.5 × 0.5 cm). They were flushed with normal saline, fixed in 10% buffer formalin, dehydrated, cleared with xylene and embedded in paraffin. All samples were blocked by paraffin. Then, sections were cut at 6 μm thickness by a rotary microtome (Leica®) and mounted on a glass slide and stained with Hematoxylin and Eosin (H&E). For ultrasonographic evaluation, samples were cut in 6 × 6 cm pieces and they immersed in normal saline. Then ultrasonography was performed using an 8 MHz probe. The external layer of rumen was near to foot print of probe and by moving the probe near and far to these segments, ultrasonography at the highest resolution possible in the focal zone was obtained. The procedure was recorded.

![Figure 1](image1.png)

**Figure 1**

Histological structure of dorsal blind sac. Lumen (L), Epithelium (E), Villi (Vi), Lamina properia (LP), mucosal and submucosal layer (ML- SM), Muscular Tunica (MT), H&E, ×40.

![Figure 2](image2.png)

**Figure 2**

Histological structure of ventral blind. Lumen (L), Epithelium (E), Villi (Vi), Lamina properia (LP), mucosal and submucosal layer (ML- SM), Muscular Tunica (MT), H&E, ×100.
through all of the stages, digitally. Then, evaluation and implementations of ultrasound and histological images of each specified area in rumen were confirmed. Mucosal and submucosal layers, and muscular and serosa layers were measured in all samples in 5 parts of each slide by using the software of Image-J 1.47. In ultrasonogram, mucosal, submucosal, muscular, and serosa layers were evaluated in three points. Average, standard deviation, minimum and maximum for each of the measured parameters were reported. The normality of data with the help of Kolmogorov- Smirnov, Shapiro-Wilk and QQ plat charts were reviewed and approved. Histomorphometric correlation and ultrasonographic data based on the Pearson correlation coefficient with a significant level of \( p < 0.05 \) were evaluated.

**Acknowledgements**

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**Author contributions**

Conceived and designed the experiments: A. M. Performed the experiments: M.M. Contributed reagents/materials/analysis tools and wrote the
Table 2
Ultrasonographic measurements of rumen wall.

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<th>Minimum (mm)</th>
<th>Maximum (mm)</th>
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References


Histomorphogenesis of pancreas in ostrich embryo (Struthio camelus)

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Keywords
ostrich, histology, pancreas, development

Abstract
The present study was carried out to determine the development of pancreas in ostrich embryo. For this purpose ostrich embryos obtained on 10, 13, 16, 22, 26, 30 and 36 days of prehatching and 1 day of post hatching life. The specimens were stained with haematoxylin eosin [H&E], Gomori and Toluidine blue, and then were explored by light microscopy. The Results showed the primitive pancreas near the liver and duodenum on day 13. Histological observations showed that the pancreas was consisted of undifferentiated epithelial cells, connective tissue, non-organized ducts and blood vessels. On day 16, pancreas was composed of two dorsal and ventral lobes with the same structure of day 13. The zymogen granules were evident in developed acini on day 22. By aging the level of connective tissue and ducts were decreased and the acinar cells were increased. Pancreatic islets were determined on the day 1 after hatching.

Abbreviations
H&E : Haematoxylin and Eosin
hr : Hour
M : Molar
μm : Micrometer

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Introduction

In origin, the pancreas develops from two separate primordial buds. The ventral pancreatic bud arises from the hepatic diverticulum and the dorsal pancreatic bud arises from the duodenum [2]. The cells of the pancreatic buds proliferate and give rise to the ducts and secretory acini. The endocrine portion of the pancreas develops from some epithelial cells which lose their connection with the duct system [10].

The avian pancreas is composed of dorsal, ventral, third and splenic lobes [with species differences]. It is divided to four lobes in chickens and quails [13] but in other birds such as duck, three lobes are identified [12]. The ostrich pancreas is composed of dorsal, ventral and splenic lobes. The cranial part of dorsal lobe is known as splenic lobe [14].

The exocrine part of pancreas in avian is similar to other animals, but there is no centro-acinar cells in chicken. Although infrequently some of these cells have been reported in starling and domestic goose [13]. The major islet cells include alpha [α], beta [β] and delta [δ] cells. The endocrine cells can be identified in small numbers between the islets [1].

Results

At the day 10 of incubation there was not any pancreatic tissue, but on day 13 it was determined near the liver and duodenum. Thus, the primary pancreas is formed between days 10 and 13 of incubation. The primordial pancreas was connected to the peripheral mesenchymal tissue and histologically was contained of undifferentiated epithelial cells and connective tissue. On day 16 of prehatching incubation, the pancreas had two dorsal and ventral lobes and was composed of developing exocrine parts, connective tissue, developing ducts and blood vessels (Figure 1).

On day 22, the structure of pancreas was similar to that in day 16. However, some mesenchymal tissue and the lobes were separated by narrow spaces. In semithin sections, the zymogen granules were rarely seen in exocrine cells (Figure 2).

On days 26 and 30 of incubation, the lobes were separated and the dorsal lobe was larger than ventral lobe. The acini were developed and the

![Figure 1](image)
Pancreas at day 16 of prehatching development. DP: dorsal part of pancreas, VP: ventral part of pancreas, MT: mesenchymal tissue, Li: liver tissue, black arrows: ducts or vessels, white arrow: space between two parts of pancreas [H&E].

![Figure 2](image)
Pancreas on day 22 of prehatching development. CT: connective tissue, black arrows: mitotic cell, white arrow: zymogen granules [Toluidine blue staining].

![Figure 3](image)
Histological structure of pancreas on day 1 after birth, A: Alpha islets, B: Beta islets in splenic lobe of pancreas [Gomori's staining].
mesenchyme tissue had significantly decreased.

By day 36, the exocrine part had been completed and the zymogen granules were increased by aging (Figure 4). One day after hatch, the endocrine part was determined by H&E stain and more of the islets were concentrated in dorsal and splenic lobes (Figure 3).

With Gomori staining by day 36, the scattered alpha cells were determined between the acini (Figure 5). The typical alpha and beta islets were obvious on day 1 after hatching, and the islets were sporadic in ventral lobe. The alpha islets with pink cells were larger and more numerous than beta islets with blue cells (Figures 6 and 7).

Discussion

The pancreas develops as dorsal and ventral endodermal outgrowth of distal foregut. The initial ventral bud arises from the hepatic diverticulum after dorsal bud [10]. In this study, primary pancreas determined between day 10 and 13 of incubation. By day 13, dorsal lobe appeared near the developing liver and by day 16, the ventral lobe was developed. In the chicken, the dorsal bud has been reported to appear by day 5 and the ventral bud has been determined on day 7 of incubation [8]. In the rodent, pancreatic bud appears at the middle stages of gestation [4]. For example the dorsal bud in rat, has been observed on day 11 of gestation (20 somite stage), in mouse on day 9.5,

Figure 4
Histological structure of pancreas on day 1 after birth. Black arrows: mitotic cell, white arrows: zymogen granules, R: RBC, PD: pancreatic duct [Toluidine blue staining].

Figure 5
Histological structure of pancreas tissue on day 36 of prehatching development. A: acini, PD: pancreatic duct, arrows: eosinophilic alpha cells [Gomori's staining].

Figure 6
Histological structure of Alpha islet of pancreas on day 1 after birth. Black arrows: scattered Alpha cell [Gomori's staining].

Figure 7
Histological structure of Beta islet of pancreas on day 1 after birth. Black arrows: Beta cell [Gomori's staining].
and in rabbit on day 11 of embryonic development. In rat, the ventral part of pancreas has been observed to develop about 12 hr later than dorsal part (28-30 somite stage) [3,16,15]. In this study on day 22, the lobes were separated with a narrow space. In chicken, the dorsal and ventral lobes are distinguished on day 9 and all the pancreatic lobes are demonstrated to be present by day 12 of incubation [8].

We noticed that between days 13 and 16 of incubation, the pancreas consisted of the undifferentiated epithelial cells, non-organized ducts, blood vessels and lots of mesenchyme connective tissue. Zymogen granules and mitotic cells were obvious in developing acini on day 22. By aging, the level of connective tissue and ducts were decreased and zymogen granules were increased. Mitosis was observed in all embryonic stages. In chicken, between days 5 and 7, the pancreas is composed of epithelial cells in mesenchymal tissue and between days 9 and 12, the ducts appear in connective tissue [8]. In rat by day 13, the pancreatic tissue consists of undifferentiated epithelial cells with high volume of mesenchyme and on day 19, most of epithelial cells are acinar cells with zymogen granules and ducts [3]. In rabbit, on day 12 of gestation, the pancreatic cords with thin lumen appear and the ductal and exocrine cells have mitotic activity. The cells have many free ribosomes and rough endoplasmic reticulum [15]. As expected, from fetal to newborn, the exocrine secretory units increase and the level of blood vessels and connective tissue decreases [9].

In this study, on day 1 after hatching the islets were appeared by haematoxylin & eosin [H&E] and Gomori’s stain. In chicken, by day 5, the endocrine cells are detectable with immunohistochemistry [8]. In quail the endocrine part includes beta, alpha and mixed islets and they are centralized in splenic and third lobes [13]. The pancreatic islets in goose are also composed of alpha, beta and mixed islets [11]. In rat, the glucagon cells are determined by day 11 and insulin cells are detect on day 12.5 by immunohistochemistry [3]. In rabbit, insulin and glucagon immunoreactive cells are infrequently detected on day 13 of embryonic development [15]. In human, insulin immunoreactive cells are found on week 7 of development [5]. Thus, according to other studies, immunohistochemical staining is suitable for detection of the endocrine cells in initial stages. In ostrich, on day 1 after hatching the islets were determined by routine examination with haematoxylin & eosin and Gomori’s staining. More islets were concentrated in dorsal and splenic lobes and the alpha islets were higher in number and larger than beta islets.

Materials and methods

In this study, twenty four ostrich embryos on days 10, 13, 16, 22, 26, 30 and 36 of prehatching and day 1 of posthatching life were used. The pancreas was separated and fixed in 10% buffered formalin and Bouin’s solution for histological examinations. The fixed specimens were dehydrated with ethanol, cleared in xylene, embedded in paraffin wax, cut at 5-6 μm thickness, and stained with haematoxylin eosin [H&E] and Gomori [7].

For semi-thin sections, small specimens of pancreas were fixed in 2% glutaraldehyde in Na-cacodylate buffer, pH 7.4 for 1-2 hours. They were washed in the same buffer. For secondary fixation, the samples rinsed in 1% osmic acid in 0.1M Na- cacodylate buffer for 50-60 minutes. Then the sections were washed and dehydrated in ethanol. Propylene oxide was used for infiltration of resin in tissue and the samples embedded in Araldite-Epon mixture. Semi-thin sections [1μm in thickness] stained with Toluidine blue [6].

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Author contributions

Conceived and designed the experiments: Z.S. Performed the experiments: M.A. Analyzed the data: Z.S., M.A., H.G.A. Research space and equipment: Z.S. Contributed reagents/materials/analysis tools: Z.S. Wrote the paper: Z.S., M.A.

Conflict of Interest

None of the authors of this paper have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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Effect of sex on histological and histochemical structures of interdigital sinus in adult Bakhtiari sheep of Iran

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Keywords
histochemical, histology, interdigital Sinus, sex, sheep

Abstract
The aim of this study was to investigate the effect of gender on the histological and histochemical structures of various anatomical regions of the interdigital sinus in the Iranian native sheep. Tissue samples from different anatomical regions of each sinus were obtained from 15 female and 15 male adult Bakhtiari sheep. The sections stained with H&E, Periodic acid- Schiff, Alcian blue, Verhoeff’s, Masson’s trichrome, and Gomori’s method. The sinus was covered by skin and fibrous capsule. The sinus contained descending part, flexure, ascending part, excretory duct and orifice. The sebaceous glands were simple branched acinar and the sweat glands were simple coiled tubular type. The secretory caps were observed in the secretory cells protruded into the lumen. The various histological and histochemical structures of the interdigital sinus showed no considerable differences among various anatomical regions of the right and left sinuses. Also, no significant sex-based differences were found. The surface epithelial cells of the apocrine glands and their secretion contained both neutral and acidic glycosaminoglycans. It is concluded that the general histological and histochemical structures of the interdigital sinus in Bakhtiari sheep were similar to those of other native sheep, but there were also some differences.

Abbreviations
H&E: Hematoxylin eosin
PAS: Periodic acid- Schiff
AB: Alcian blue
Introduction

Sebaceous glands arise from epidermal buds of the skin. In defined body regions of some domestic species especially in the interdigital sinuses of sheep, well developed accumulations of sebaceous glands are obvious [1]. Interdigital sinuses are found on the forelimbs and hindlimbs of sheep of both sexes [2], and located between the digits just above the hoofs [3]. Numerous apocrine sweat glands are associated with the interdigital pouches of sheep [4]. These pouches possess waxy secretion which is discharged through a single opening above the hoofs and serve as a trail marker [2].

The sheep population in Iran is 50 million, belonging to 20 breeds. More than 96% of Iranian sheep are fat-tailed. One of the main Iranian fat-tailed and native breeds is Bakhtiari, which is encountered in the western and southwestern regions of the country especially in Charmahal va Bakhtiari province [5, 6].

For elucidation of the histology and histochemistry of the interdigital sinus, investigations have been carried out in different native sheep, such as Lori [7], Tuj [8], Dubska pramenka [9], Kivircik [10], and Yankasa [11]. However, there is no information regarding gender effects on the histology and histochemistry of the interdigital sinus in Bakhtiari sheep. The aim of the present study was to describe the histological and histochemical properties of the Bakhtiari interdigital sinus and to reveal if these properties are sex dependent, and to compare the findings with those made in other sheep breeds.

Results

In this study, the histology and histochemistry of the interdigital sinus showed no considerable differences among various anatomical regions of the right and left interdigital sinuses. Also, no significant differences were found between the male and female Bakhtiari sheep. The interdigital sinus of Bakhtiari sheep was found on the forelimb and hindlimb of both sexes.

The shape of the interdigital sinus in this native sheep was curved tube-tobacco pipe-shaped and contained descending part or fundus, flexure, ascending part, excretory duct or neck and orifice (Figure 1A). The interdigital sinus of the forelimb (3.5 × 1.77 cm) was somewhat wider and longer than those of the hindlimb (2.88 × 1.55 cm). The sinus was located in the interdigital space and covered with a capsule of dense connective tissue and skin (Figure 1B).

Histologic examination revealed that the interdigital sinus capsule was composed of moderate thick dense connective tissue and contained nerve bundles, adipose tissue (Figure 2A), blood vessels, collagen (Figure 2B), elastic (Figure 2C), and reticular fibers (Figure 2D). The skin of the interdigital sinus was consisted of the epidermis and dermis. The epidermis was composed of keratinized stratified squamous contained stratum basale, stratum spinosum and granulosum. The stratum granulosum was not always well delineated. The melanin granules were observed in stratum basale (Figure 3A).

Figure 1
The interdigital sinus in the Bakhtiari sheep. A) The sinus was curved tube-tobacco pipe-shaped and contained descending part (D), flexure (F), ascending part (A), excretory duct (E) and orifice (O). The lumen of the sinus filled with hairs (arrowheads) and glandular waxy secretion (arrows). B) The sinus located in the interdigital space between the digits is covered with a capsule (C) of dense connective tissue and skin.
The dermis was contained hair follicles, sebaceous glands, nerve plexus, arrector pili muscles and numerous large apocrine sweat glands (Figure 2A, 2B, 2D).

Numerous hairs with different sizes were associated with the interdigital lumen but they were absent in excretory duct. The interdigital lumen was filled with hairs and glandular waxy secretion of the sinus (Figures 1A, 2A). Among sinus regions, the highest and lowest hair follicle densities were found respectively in descending part and orifice. The sebaceous glands in the interdigital sinuses were simple branched acinar types. The sebaceous glands were always associated with hair follicles and located just above the apocrine sweat glands (Figure 2A, 2B). The sebaceous glands were larger and more branched in excretory duct of the interdigital sinus. The arrector pili muscles were found around the hair follicles. These bundles of smooth muscle cells were inserted on the dermal sheath and attached to the dermis of the interdigital sinus (Figure 2A).

The sweat glands with different shapes and sizes in the interdigital sinuses were simple coiled tubular type. These large apocrine glands were mostly below the sebaceous glands, just between the hair follicles and the boundary of papillary and reticular layer of dermis (Figures 2A, 2B, 2D). The secretory caps that indicated their secretory activity, were observed in the free apical cytoplasm of secretory cells protruded into the lumen (Figures 2B, 3B). The maximum sweat gland frequency was observed in descending part of sinus.

The secretory cells of sweat glands were lined-
Effect of sex on histological and histochemical structures of interdigital sinus

**Figure 3**
A) The epidermis (E) of the interdigital sinus of the Bakhtiar sheep was composed of stratum basale (b), stratum spinosum (s), and stratum granulosum (arrow). The melanin granules (arrowheads) observed in stratum basale, D: dermis, hematoxylin and eosin. B) The sweat glands of the sinus were simple coiled tubular type. Their secretory cells surrounded by the myoepithelial cells (arrows), the secretory caps observed into the lumen (arrowheads), H&E.

with a simple to stratified cuboidal epithelium. The secretory cells surrounded by one row of myoepithelial cells and a thin layer of connective tissue fibres. Among sinus regions, the myoepithelial cells were more numerous in excretory duct (Figure 3B).

All epithelial cells of the apocrine glands and their secretion reacted positively to periodic acid Schiff (PAS) (Figure 4A), and alcian blue stains (Figure 4B).

**Discussion**

In the present study, the histology of the interdigital sinus showed no significant differences according to sex which is in agreement with the results reported by Aslan et al. [8] in Tuji sheep and Atoji et al. [13] in Japanese sorrow, but Abbasi et al. [7] reported sex histological differences in adult Lori sheep.

The interdigital sinus of Bakhtiar sheep was found on the forelimbs and hindlimbs of both sexes. This finding is in agreement with what was reported for some native sheep breeds [7-10]. Janicki et al. [14] reported that the interdigital sinus was found only in the hind feet of the roebuck.

The interdigital sinus in Bakhtiar sheep was

**Figure 4**
A) PAS-positive material (arrows) is present in the secretory epithelial cells of the apocrine glands and their secretion (WS), L: interdigital lumen, PAS. B) AB (+) luminal apocrine epithelial cells in the interdigital sinus of Bakhtiar sheep (arrowheads).
curved tube-tobacco pipe-shaped. Similar results were also reported by Abbasi et al. in Lori’s sheep [7], Aslan et al. in ‘Tuji’ sheep [8], Avdic et al. in Dubska pramenka [9], and Demiraslan et al. in Kivircik sheep [10].

Like other native sheep [7, 9], the interdigital sinus of the forelimb of Bakhtiari sheep (3.5×1.77 cm) were also wider and longer than those of the hind feet (2.88×1.55 cm). Aslan et al. [8] reported no sex differences between forelimbs and hind feet in Tuji sheep. Although Abbasi et al. [7] divided the interdigital sinus into the secretory and excretory units, Avdic et al. [9] defined the interdigital sinus as the fundus, collum (neck) and corpus. Meanwhile, Karahan et al. [15] divided the interdigital sinus into neck, flexure and body, while Demiraslan et al. [10] described this sinus with the terms corpus, flexure, excretory duct and orifice. In this study, the interdigital sinus was divided into fundus or descending part, flexure, ascending part, neck or excretory duct and orifice.

The interdigital sinus of the Bakhtiari sheep was covered with a capsule of dense connective tissue and skin which accords with the findings of other authors [7-10, 14-15]. Janicki et al. [14] reported that in the roebuck the interdigital sinus was situated inside the loose connective tissue.

The sinus capsule was consisted of blood vessels, adipose tissue, nerve bundles, reticular, elastic and collagen fibers. Abbasi et al. [7] reported that the capsule was contained of blood vessels, adipose tissue, nerve bundles, and bundles of collagen in Lori’s sheep. The skin of the interdigital sinus of the Bakhtiari sheep was consisted of the epidermis and dermis, which corresponds to the findings of other authors [7-10, 14-15].

The epidermis was composed of keratinized stratified squamous including stratum basale, spinosum and granulosum. Abbasi et al. [7] reported only stratified squamous epithelium with a prominent keratin layer in Lori’s sheep. In this study, the dermis was composed of hair follicles, sebaceous glands, nerve plexus, arrector pili muscles and numerous large apocrine sweat glands which are similar to previous findings [3, 7-10]. Unlike Tuji sheep [8], lymph follicles were not found in the dermis of the interdigital sinus in Bakhtiari sheep.

Some researchers [7, 9-10, 14-15], reported that the lumen of the interdigital sinus was filled with secretory material. This finding is in agreement with our results. These waxy secretions may serve as a trail marker [2] or play a role in the production of pheromones [16] or odoriferous signals in the social life of animals [8].

In the Alcian blue staining, positive reaction was observed in surface epithelial cells of the apocrine glands and their secretion. Similar results were also reported by Abbasi et al. in Lori’s sheep [7], Demiraslan et al. in Kivircik sheep [10], and Janicki et al. in roebuck [14]. This reaction indicated that the apocrine secretion consisted of neutral glycosaminoglycan.

It was determined that the highest hair follicle densities were found in descending part of the interdigital sinus, while in Dubska pramenka the hair follicles were more numerous around the orifice of the interdigital sinus [9]. In the present study, the wall of the interdigital sinus had well-developed sebaceous glands and numerous large apocrine sweat glands that were in matching with results of the literature [3-4, 7, 9-10, 14-15].

Janicki et al. [14] reported that the sebaceous glands are compound alveolar, but it may also be simple alveolar [7]. The observations made in this study exposed the sebaceous glands of interdigital sinus in Bakhtiari sheep were from branched acinar type.

In the present study, the arrector pili muscles were found around the hair follicles of the interdigital sinus which agrees with the findings of Abbasi et al. in Lori’s sheep [7]. The sweat glands of interdigital sinus in Bakhtiari sheep were simple coiled tubular type which is similar to previous findings [7, 14]. The secretory cells of these apocrine glands surrounded by one row of myoepithelial cells which is according to the findings of Abbasi et al. in Lori’s sheep [7].

The histological and histochemical features of the interdigital sinus of the Bakhtiari sheep were similar to those in the other native sheep except the special features for the capsule; with the connective tissue fibres, epidermis; which composed of stratum basale, spinosum and granulosum, the absence of lymph follicles in the dermis and the presence of more numerous hair follicle in descending part of the interdigital sinus. There were no significant sex differences on the histology and histochemistry of the interdigital sinus.

Materials and Methods

A total of 120 interdigital sinuses in the forefeet and hind feet of the 30 adult, healthy Iranian Bakhtiari sheep (15 females and 15 males) aged 1–2 years, were examined. The feet of the Bakhtari sheep were obtained from the Shahrekord
Municipality Slaughterhouse. The interdigital sinuses were removed from the subjects and the samples were taken from different regions of the sinuses. Tissue samples from different anatomical regions of each interdigital sinus were fixed in 10% neutral buffered formaldehyde for 48 h and processed to embed in paraffin.

The serial sections (5 μm) were stained with haematoxylin eosin for general histological observations and special techniques: Masson’s trichrome (for collagen fibres), Verhoeff’s (for elastic fibres), and Gomori’s methods (for reticular fibres). To investigate the chemical character (pH) of the secretion material in the epithelial cells, Alcian blue reaction (pH, 2.5) was employed to determine acidic mucosubstance and Periodic acid-Schiff (PAS) was used for determining neutral mucosubstances [12]. The histological and histochemical studies on stained sections were carried out by light microscopy (Olympus BX50, Japan).

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Author Contributions

Conceived, designed and performed the experiments: B. Mobini. Contributed reagents/materials/analysis tools: V. Ranj kesh Adermanabadi.

Conflict of Interest

The authors have no conflict of interest to declare.

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Effect of thiamine and vitamin C on tissue lead accumulation following experimental lead poisoning in Cyprinus carpio

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Keywords
lead, tissue, common carp, thiamine, vitamin C

Abstract
The present study was conducted to evaluate the possible ameliorative effects of vitamin C and thiamine on lead accumulation in kidney, liver, muscle, brain and gill of experimentally lead-poisoned common carp. At the beginning of the experiment, fish (n=120) were divided into 4 groups randomly with group 1 being considered as the control group. Groups 2, 3 and 4 were exposed to lead acetate (5 mg/L, 15 days); groups 3 and 4 received vitamin C (500 mg/kg feed) and thiamine (50 mg/kg feed) during lead acetate exposure, respectively. Following this, it was observed that lead exposure caused a significant ($p < 0.05$) increase in lead content in all examined tissues of fish in group 2 in comparison to control group. It was also found that thiamine supplementation slightly decreased the augmented levels of lead in the muscle, brain and gill tissues, which was not significantly different from that of the control group. Similarly, vitamin C supplementation reduced the augmented concentrations of lead in the muscle to the levels that were not significantly different from that of the control group. Based on the present results, neither thiamine nor vitamin C was effective in providing a significant reduction of tissue lead burden in groups 3 and 4 as compared to group 2. Thus, monotherapy with such vitamins cannot be proposed as a suitable therapeutic approach for the effective reduction of the tissue lead burden in common carp. However, further investigations using other dosing regimens of each vitamin or combined treatment with chelators are required to reach such a conclusion.

Abbreviations
Pb: Lead
DMSA: DiMercaptoSuccinic Acid
Na$_2$Ca-EDTA: Sodium Calcium Edetate

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**Introduction**

Lead (Pb) is an environmental pollutant and its pollution has increased drastically in the last century resulting in lead poisoning becoming one of the greatest concerns of the world [1, 2]. Due to its physical and chemical properties, lead has been utilized in many industries; as a result, through industrial discharges, sewages, batteries etc., lead has found its way into fresh waters [3-6]. Having an accumulative behavior, exposure to prolonged low levels of heavy metals can induce their high accumulation in tissues without causing mortality in fish [6-8].

Several adverse effects of lead toxicity including neurological, hematological, gastrointestinal, reproductive, circulatory and immunological dysfunctions, as well as its carcinogenic effects have been reported [9, 10]. Since heavy metal pollutants build up in food chain, consumption of seafood may pose a high risk, especially to human consumers [6, 11]. Although the currently approved approach against lead toxicity is using chelating agents which will bind with and withdraw lead from lead-burdened tissues, some toxic effects of such agents necessitate research on alternative therapeutic approaches, particularly using natural compounds [12-14]. Vitamin C is a free radical scavenger and is used as a prophylactic agent against lead induced oxidative stress by quenching reactive oxygen species. It has been also proposed that vitamin C could have a chelation capacity for lead [9, 15, 16]. Thiamine can also make readily excretable complexes and expedite lead elimination [13, 15].

The present work seeks to evaluate the effects of thiamine and ascorbic acid on reducing lead accumulation in some tissues of experimentally lead-poisoned common carp. To our knowledge, this is the first study concerning the effect of thiamine and ascorbic acid on the tissues lead content in carp. These studies may be helpful in providing practical approaches against lead toxicity in polluted freshwaters in order to diminish some seafood hazards threatening animals and human health.

**Results**

Mortality was not observed among experimental groups during the experiment. Lead accumulation in various tissues following lead acetate administration is shown in figures 1 and 2 as mean ± SEM. Lead exposure caused a significant ($p < 0.05$) increase in the lead content of the kidney, liver, brain, muscle and gill of fish in group 2 in comparison to the control group. The highest tissue lead accumulation following lead acetate treatment was observed in the kidney followed by the liver, muscle, gill and brain (Figures 1 and 2).

As the results show, neither thiamine nor ascorbic acid caused a significant declining effect on tissue lead burden as compared to group 2. Thiamine supplementation in group 4 decreased the augmented levels of lead in muscle, brain and gill tissues to the levels that were not significantly dif-
different from that of the control group. In a similar manner, ascorbic acid supplementation decreased the augmented concentrations of lead in the muscle, but not in other examined tissues, to the levels that were not significantly different from that of the control group (Figures 1 and 2).

Discussion

The existence of high levels of heavy metals in food animals is of great concern. Lead exposure has been shown to decrease vitamin content in various tissues and administration of some vitamins has also been proposed to reduce the toxic symptoms of lead [17, 18]. The use of dietary constituents with possible chelating properties may be considered as a potential approach to alleviating health problems related to toxic metal exposure. In this experiment, the possible declining effects of ascorbic acid and thiamine on tissue levels of lead in Pb-exposed carp have been investigated.

Accumulation of heavy metals in fish organs may be affected by several parameters such as exposure dose and time, route of administration, age of fish, interaction with other metals, water chemistry and metabolic activity of the fish [19]. In fish, gill and intestine can be considered as the main sites of Pb uptake [20, 21]. Based on the present results, the order of Pb accumulation in different organs of fish following lead acetate exposure was kidney > liver > muscle > gill > brain. The organ distribution of lead in our study is to some extent reminiscent of previous work documented in carp [22]. Heavy metals affinity for liver and kidney may be attributed to the higher metabolic activity of these organs and their detoxifying role. However, it has been shown that the highest concentration of lead in lead-exposed Tilapia zilli was observed in the gill followed by liver, brain and then muscle [23].

Many of the chelating agents used to reduce lead content of tissues have not been approved in food animals [24]. Both thiamine and vitamin C have been suggested to have chelating capacities for lead; they can also reduce intestinal lead absorption. Vitamin C could even act as a free radical scavenger which magnifies its importance against lead-induced tissue damages [9, 12, 15, 25]. It has been previously reported that ascorbic acid and thiamine partly exhibited protection against Pb-induced tissue biochemical alterations in carp as manifested by alleviation of toxicant-mediated rise in lipid and protein oxidation markers and metabolic enzyme activities in some organs [26].

Our findings indicate that thiamine supplementation decreased the augmented levels of lead in muscle, brain and gill tissues to the levels that were not significantly different from that of the control group. However, thiamine administration could not significantly reduce the tissue lead burden as compared to group 2. This agrees with the observations of Coppock et al. (1991) showing that thiamine alone was not effective in reducing Pb concentration while administration of Na2, Ca-EDTA alongside thiamine was proven as an effective treatment for experimentally induced environmental lead poisoning in cattle [27]. Moreover, the low efficacy of thiamine alone on the retention of lead in tissues has also been reported in mice [25] and goat [28]. In contrast to our findings in common carp, Ghazaly (1991) showed the effectiveness of thiamine in preventing lead deposition in blood, kidney, liver, brain and muscle of lead-exposed Tilapia zilli [29]. Furthermore, it has been reported that thiamine administration could decline the blood and ovaries’ lead content in subacute lead poisoning in sheep which was not the case with the spleen and brain [13].

Indeed, ascorbic acid supplementation decreased the augmented concentrations of muscle lead to the levels that were not significantly different from that of the control group; however, it did not have the same effect on other examined tissues of carp. Although the efficacy of vitamin C in providing effective reduction of oxidative stress has been reported in lead-exposed rats, its effect on reducing the lead burden in liver, kidney and brain was not significant. Besides, co-administration of vitamin C during chelation with meso-2,3-dimercaptosuccinic acid (DMSA) or monoisoamyl DMSA had little or no additive effect on the depletion of lead compared to the effect of chelators alone [17]. However, Vij et al. (1998) indicated that vitamin C supplementation in lead-exposed male rats significantly reduced the level of lead in liver, kidney, and blood [18]. Ascorbic acid increased urinary elimination of lead and reduced the hepatic and renal lead burden in rats [30]. It has been proposed that the effect of vitamin C on lead absorption and excretion may be more obvious in low-exposed subjects with higher vitamin C supplementation. On the other hand, in human and animals exposed to high concentrations of lead,
the decline of lead burden following vitamin C administration is less significant [31]. As noticed above, some variations exist in the literature concerning the effects of thiamine and vitamin C on the tissue lead accumulation that might be associated with the differences in animal species, sample size, utilized doses, route and timing of exposure, experimental situations and procedures or other unknown factors.

In view of our results, it can be suggested that neither thiamine nor ascorbic acid were effective in providing significant reduction of tissue lead burden in lead-exposed common carp. Thus, monotherapy with such vitamins cannot be proposed as a suitable therapeutic approach for an effective reduction of body lead burden in common carp. However, further investigations using other dosing regimens of each vitamin or combined treatment with chelators are required to reach such a conclusion.

**Materials and methods**

**Chemicals**

Lead acetate was supplied by Merck (Darmstadt, Germany). Thiamine and ascorbic acid were purchased from Hakim Co. (Tehran, Iran) and Chemifarma Co. (Tehran, Iran). Sulfuric acid and nitric acid were supplied by Sigma (St. Lewis, MO, USA) and Merck (Darmstadt, Germany).

**Experimental design and sampling**

Healthy common carp (Cyprinus carpio; total, n=120), weighing 100 ± 10 g (mean ± SD), were obtained from a local commercial farm. Fish were divided randomly into four groups of 30 each and were held in four glass aquaria, each containing 250 L of fresh water. Fish were acclimatized for 15 days prior to the commencement of the experiment and were fed daily with commercial fish food at 2% total body weight at a fixed time. Physicochemical conditions of the water during the experimental period were dissolved oxygen, 5.5–6 ppm; temperature, 25 ± 1 °C; and pH, 7 ± 0.5. The photoperiod was a 12:12 light/dark cycle. The water in the aquaria was renewed every 48 h. The fish in group 1 were reared in normal freshwater and served as the control group. Group 2 received lead acetate (5.0 mg/L) while group 3 were exposed to lead acetate (5.0 mg/L) and received vitamin C (500 mg/kg feed). Also, group 4 received lead acetate (5.0 mg/L) and thiamine (50 mg/kg feed).

At the end of each exposure (15 days), ten fish of every aquarium were randomly selected and euthanized using tricaine methanesulfonate. Then, the liver, kidney, muscle, brain and gill were quickly removed, cleaned free of extraneous material and washed with physiological saline. Tissue samples were frozen in liquid nitrogen and stored at −70°C until analysis.

**Analysis and measurements**

Tissue preparation was performed using a slight modification of the wet-ashing technique [32] as described by Najarezhad et al. (2010). Weighed pieces of tissues were digested in a 1:1 mixture of 98% sulfuric acid and 70% nitric acid. Ten milliliter of acid mixture per gram of tissue wet weight was used. Samples were heated at 120°C for 4 h, with acid mixture added as needed in a drop-wise manner to prevent char-ring until the organic matter was completely destroyed and finally the volume of solution reached 50 ml.

Lead concentrations in prepared samples were determined (in Toxicology Laboratory of Imam Reza hospital, Mashhad, Iran) by atomic absorption spectrophotometer (Perkin-Elmer3030) at 283.3-nm wavelength using a graphite furnace. The limit of detection for this analysis was 5 ng/g and recovery for spiked samples was >90%. The results were expressed as μg/g wet weight of tissue samples.

**Statistical analysis**

All experimental values are represented as mean ± standard error of the mean (SEM). All results were analyzed using one way analysis of variance (ANOVA), followed by Bonferroni multiple comparisons test. The level of significance was set at \( p < 0.05 \). All calculations were performed using SPSS/PC software.

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**Author Contributions**

Conceived and designed the experiments: D.S., H.B. Performed the experiments: K.N., D.S., H.B. Analyzed the data: H.B. Contributed reagents/materials/analysis tools: H.B. Wrote the paper: K.N., H.B.

**Conflict of Interest**

None.

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Fibrosarcoma in a Goldfish (Carassius auratus): a Case report

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Keywords
fibrosarcoma, goldfish

Abstract

Neoplasms in fishes are generally less aggressive than neoplasms in mammals and are most commonly discrete and focal. Although tumors were originated from connective tissue are frequently seen in the fishes, but dermal fibrosarcoma is rarely found in fish. A moribund four years old Goldfish (Carassius auratus), was referred veterinary hospital of Shahid Chamran University of Ahvaz with an exophytic non-ulcerated mass near the caudal peduncle. Microscopic examination of the mass revealed proliferated spindle cells and the interlacing, loose and eosinophilic bundles. For differential diagnosis, Masson’s trichrome staining was performed and the fibers stained blue. According to histopathological and histochemical results, tumor identified as fibrosarcoma. It is the first report of histopathological features of cutaneous fibrosarcomas in a Goldfish in Iran.

Abbreviations
WDSV: walleye Dermal Sarcoma Virus

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Fibrosarcoma in a Goldfish

Introduction

Tumors of connective tissues are evaluated in subclassification of spindle cell tumors. Dermal fibroma and fibrosarcoma are infrequently found in fishes, however, tumors of fibrous connective tissues are frequently seen in all types of mesenchymal tumors of fishes. The dermal tumors are usually raised from the surface of the trunk, head and fins. It is substantiated that the tumors are likely found in association with environmental factors and infectious agents in farmed and wild fishes [8].

Fibroma is a benign tumor arising from fibroblasts, and fibrosarcoma is malignant equivalent with high cellularity. Proliferated fibroblasts are often arranged in irregular cords, and whorls. No clear etiology was established and the histogenesis of some of these tumors is ambiguous. Different types of fibrosarcoma have been reported in a number of species, including Goldfish (*Carassius auratus*) with three mass on the right side of the body, yellow perch [*Perca flavescens*] with two tumors on the right flank near the dorsal fin, and fork tailed catfish (*Hemiarius dioctes*) with multiple tumors in oral cavity, operculum and caudal peduncle [1; 3; 5]. Bowser et al (2005) have confirmed the presence of walleye dermal sarcoma virus (WDSV) by PCR and suggested that the virus is horizontally transmitted within the walleye population of the lake during spring when the adult fish migrate in streams for spawning [3]. Dennis and Diggles (2015) reported the histopathological features of multiple orocutaneous fibrosarcomas in a warrior catfish [*Hemiarius dioctes*] [5]. This report is related to clinicopathological features of cutaneous fibrosarcomas in a Goldfish in Iran.

Case description

A four-year old Goldfish with an exophytic non-ulcerated mass near the caudal peduncle were referred to the Aquatic Animals Health Department of Shahid Chamran University of Ahvaz. The owner has observed the tumor one year ago, but the fish became depressed and anorectic in the 1-2 week before presentation. Because of lethargy and unbalance swimming, the prognosis was poor and no therapeutic procedure proposed. The fish was euthanized and necropsy was carried out. The external surfaces, mouth, gill chambers and internal organs of the fish were clinically examined. The weight of fish was 44 grams and its length was 18.5 cm and had the enlarged abdomen, which persisted for more than 2 weeks.

Macroscopically, the mass was localized in caudal peduncle and its diameter was approximately 1.5 cm. It had a rough surface and the consistency was firm and gray-white color (Figure 1). The cut surface was firm and white.

The tumor mass was fixed in 10% neutral buffered formalin and sent to the histopathological lab. The mass was sectioned at 5 μm thicknesses from paraffin blocks and stained with Hematoxylin and Eosin (H&E) and Masson’s Trichrome. The slides were observed by light microscope.

Figure 1
Goldfish (*Carassius auratus*). A white mass is visible near the peduncle.

Figure 2
Low magnification of a histological section of fibrosarcoma in Goldfish (*Carassius auratus*). Note the interlacing bundles of connective tissue, which are in different directions (Hematoxylin and Eosin).

Microscopic examination of the mass revealed proliferated spindle cells with eosinophilic loose fibers. They were located in the interlacing pattern (Figure 2). The numbers of nuclei were high and hypercellularity was obvious. Also, pleomorphism
and anisokaryosis were identified which were characterized by different shape and size of nuclei (Figures 3 and 4). Necrosis and haemorrhage were not seen. As the entity of fibers was not detected, Masson's trichrome staining was performed and the interlacing bundles were stained blue (Figures 5 and 6). According to histopathological and histochemical results, fibrosarcoma was diagnosed.

Discussion

This report described histopathological characteristics of fibrosarcoma in a Goldfish. The existence of spindle cells and interlacing fibers were confirmed which was in accordance with other reports [1, 8]. These characteristics are seen in other lesions such as granulation tissue, fibroma, leiomyoma and leiomyosarcoma. In granulation tissue, the fibers are in one direction and between them, angiogenesis is occurred. However in this mass, angiogenesis was not seen and the direction of fibers was completely different and they had interlacing pattern. The hypercellularity, pleomorphism and anisokaryosis of proliferated cells showed malignancy. The entity of fibers was detected by histochemical staining (Masson's Trichrome). The fibers did not have a muscular entity and they were differentiated from leiomyosarcoma or rhabdomyosarcoma.

Fibrosarcoma is a rare and malignant soft tissue sarcoma. Etiology is usually unknown, but is often
suspected to involve viral or chemical factors [8]. Retroviruses are first candidates for induction of cutaneous fibroma/fibrosarcoma of the hooknose (Agonus cataphractus), dermal sarcoma of walleye (Stizostedion vitreum), and fibroma of the lip of the angelfish (Pterophyllum scalare) [4]. Walker (1969) and Yamamoto et al., (1976) demonstrated C type particles of virus in the fibrosarcoma of an American pike-perch. Duncan (1978) diagnosed ultrastructurally oncoviruses associated with swim bladder fibrosarcoma in Atlantic Salmon. Another report has also been described from wild Atlantic salmon on the western seaboard of the Atlantic Ocean [7]. Adverse environmental factors such as high population densities, high concentrations of no specific pollutants and carcinogens in water, sediments and food organisms may contribute to a further suppression of defense mechanisms [2]. In the present case, the mass was developed during approximately 1 year period from 0.5 cm to 1.5 cm in diameter. It can be suggested that carcinogenic materials in water, sediment or biota can influence the development of a benign tumor by mutation-al modification of cellular functions, resulting in a progress to malignancy. However, the exact etiology is unknown and it needs more investigation.

In conclusion, according to histopathological characteristics of the tumor, it was diagnosed as fibrosarcoma.

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**Author Contributions**

Conceived and designed the experiments, performed the experiments, and wrote the paper: Z.T.D., R.P. Analyzed the data: A.R.

**Conflict of Interest**

The authors have no conflict of interest to declare.

**References**


Histopathologic report of infestation by *Centrocestus formosanus* in Iranian grass carp and common carp

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Keywords
*Centrocestus formosanus*, grass carp, common carp, histopathology

Abstract

Flukes cause severe and lethal diseases in various animals comprising fish. Both adult and larval stages are found in fish. Centrocestiasis is an infection of the fish gills by heterophyid trematode *Centrocestus formosanus*. In summer 2014, 35 fingerling grass carp and 30 common carp weight of 6 grams were referred to the veterinary hospital of Shahid Chamran University of Ahvaz. In the wet mount of the skin, mild infection of trichodina was observed. Wet mount of grass carp gills revealed large number of parasitic cysts between gill filaments. The cysts were quite clear and contained pear-shaped parasites. In histopathological examination, filaments of gill were thick and distorted. According to the wet mount and histopathologic results, metacercariae was characterised to belong to heterophyidae, *C. formosanus*.
Introduction

Centrocestiasis is an infection of the fish gills by heterophyid trematode Centrocestus formosanus which has been described in 1924 from adult parasites recovered from natural and experimental definitive hosts in Taiwan [19].

C. formosanus is an exotic parasite native to Asia that has been introduced into warm water systems around the world. This digenean requires three separate hosts to complete its complex lifecycle. Snail, often red-rim melania Melanoides tuberculatus, is the first intermediate host that utilizes the gills of various fishes as second intermediate host [3]. The cycle is completed when a fish infected with metacercariae is eaten by a definitive host, piscivorous birds or fish-eating mammals. In the digestive tract of definitive hosts, the adult trematode develops [5]. It appears that this parasite has a low intraspecific variability since no marked morphological and biometrical differences among worms from the different zoogeographical areas were observed [24].

Metacercarial infection in fish is with the subsequent economic loss. Metacercariae may affect growth and survival, or disfigure fish so that they lose their market cost as a food or ornamental product [10].

Metacercariae have been described from brackish and marine waters in some countries in Asia, particularly from South Korea [2, 7, 25]. Mood et al. (2010) reported Centrocestus formosanus metacercarial infection in the gills of different ornamental fish species including Dwarf gourami (Colisa lalia), Goldfish (Carassius auratus), Red fin shark (Labeo erythrus) and Arowana (Osteoglossum bicirrhosum) from Iran. Also, Shoaibi Omrani et al. (2010) reported the infection with Ascocotyle tenuicollis in the imported platy fish in Iran.

The aim of this report was to present clinico-pathologic description of Heterophyidae metacercarial Centrocestus formosanus infection in the gill of cyprinids in Iran.

Case description

In summer 2014, 35 fingerling grass carp and 30 common carp with an average weight of 6 grams were referred to the veterinary hospital of Shahid Chamran University of Ahvaz, Department of Aquatic Animals Health. The clear clinical symptom was mouth breathing. According to the owner’s statements, the fish had high mortality.

Wet mount of the skin and gills were prepared and studied by light microscopy.

All fish samples submitted for histopathology were fixed in 10% buffered formalin. The gill tissues were processed routinely, embedded in paraffin, sectioned, stained with Hematoxylin and Eosin (H & E), and examined using light microscopy [13].

In the wet mount of the skin, mild infestation of trichodina was observed. Wet mount of grass carp gills revealed large number of parasitic cysts between gill filaments. The parasitic cysts in wet mount of common carp gills were low. Infected gill filaments appeared shortened, thickened and severely damaged. A clear layer of tissue was observed surrounding the cyst. Also, fusions of the filaments were observed.

The cysts were quite clear and contained pear-shaped parasites with rapid movements (Figure 1). An X-shaped excretory bladder was seen (Figure 2A). Also, they had 32 circumoral spines around the oral sucker which were arranged in two rows (Figure 2B).

Histopathologically, filaments of gill were thick, distorted, and also they were multifocal. This area was composed of proliferated cartilage and sections of parasite in the center (Figure 3A, B). These metacercarial cysts completely disrupted the normal gill morphology. According to wet mount and histopathologic results, metacercariae was characterized to be C. formosanus.

Discussion

Unlike the majority of the digeneans, C. for-
mosanus causes morbidity and mortality in many wild and cultured fish [24]. The cercariae of C. formosanus are highly pathogenic to piscine hosts because they encyst in the gills and cause respiratory problems, however this trematode is not highly pathogenic in mammals, they can cause intestinal pain, diarrhea, and chronic enterocolitis in man, or death in experimental animals if heavily infected [11].

A massive metacercariae infestation of gill with C. formosanus was the cause of farmed Cichlid fish’s mortalities [23]. Lauckner (1984) showed that only a metacercariae of digenean trematode was sufficient to kill a fish larva. A Centrocestus species was the cause of mortality in affected common carp fry in India [17] and so the mortality of fish in this report was probably due to parasitic infestation.

Thien et al. (2007) found that severity of metacercarial infection was significantly correlated with the smaller body weights in common carp, so that small fish have a higher prevalence of metacercarial infection than larger fish [29]. The fingerlings are more susceptible to trematodes infection. This is may be due to their relatively thin skin, lack of previous contact to infection [12], less gill surface area, thus, requiring fewer metacercariae to cause greater disruption of respiratory process, and having slower defense response than larger fish [14]. Differences in the age-related quantity or structure of biochemical compounds expelled by fish might be another reason for higher infection in small fish [6].

Identification of the metacercariae was based on characteristic features (Shape of cysts, existence and size of suckers, and figure of excretory blad-
Gill infestation by *Centrocestus formosanus* [27]. In this case, X-shaped excretory bladder and 32 circumoral spines help to diagnose type of metacercariae which belongs to *C. formosanus*.

The histopathological changes of the gills were chondroblastic hyperplasia of the primary lamellae and fusion of them in the affected gill filaments. They envelope the cysts composing parasite. This histopathological finding was in agreement with Mitchell et al. (2000), Mitchell et al. (2002), Blazer and Gratzek (1985) and Olson and Pierce, (1997). The passage of the cercaria into the gill tissue and formation of a metacercariae induces a reactive chondroplasia and inflammation in the gill. The lesions vastly diminish respiratory capacity.

Exposing to unfavorable conditions for example environmental factors, and stress increase the parasite infection [8]. Recently Alves Pinto et al. (2015) reported that corticosteroid therapy increased development and fecundity of *C. formosanus* in treated mice in comparison with untreated mice. Thus, immunosuppressed host may spread fluke eggs in the environment and/or show greater severity of the disease caused by heterophyids, and perhaps other intestinal trematodes. Thus, the monitoring, diagnosis and treatment are essential.

In conclusion, this report described clinico-pathologic characteristics of metacercarial *C. formosanus* infection in the gill of cyprinids in Iran. As the cysts remain for years and they are the source of infection for the definitive hosts [4], it might have important impacts on aquaculture. In order to prevent and control the infection, food safety in fish nurseries, are recommended by Phan et al. (2010), as keeping fish fry in cement or composite tanks in filtered water [28], and removal of the vector snail by using chemical molluscicides such as copper sulphate in fish ponds and circulation systems [26].

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**Author Contributions**

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**Conflict of Interest**

The authors have no conflict of interest to declare.

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Abstracts (In Persian)

Persian Abstracts

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بررسی محتوای آنکسین A1 و A2 و سیتوکین ها در سیتی سیم تجربی گوساله‌ها

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چکیده

آنکسین‌ها از خانواده پروتئین‌ها هستند که در فرآیندهای فیزیولوژیک و پاتولوژیک مختلفی در بدن، از جمله سرکوب التهاب، نقش اساسیی دارند. از آنجا که سیتوبلازم سلول‌های ایمنی مانند نوترفیل‌ها در شرایط عادی حاوی سطح بالایی از آنکسین نوع A1 است، مطالعه حاضر، جهت بررسی تغییر و همبستگی آنکسین و سیتوکین‌های التهابی در طول سیتی سیم تجربی انجام شد.

به‌عنوان مثال، تعداد نوترفیل‌ها در شرایط عادی حاوی سطح بالایی از آنکسین نوع A1 است. این سیتی سیم قابلیت از نوع A1 و A2 یا انتهار فاکتورهای ارتباطی با پلیمر‌ها از ویژگی‌های اصلی نوترفیل‌ها می‌باشد. همچنین جهت بررسی این فاکتورهای ارتباطی با پلیمر‌ها، به‌عنوان مثال در سیتی سیم تجربی که فاکتورهای ارتباطی با پلیمر‌ها در آن وجود داشت، تعداد نوترفیل‌ها شرایط عادی و به‌کارگیری دیگر متابولیت‌ها بر روی این سیتی سیم تجربی در حال حاضر در حال تحقیق است.

واژگان کلیدی: آنکسین، سیتوکین‌ها، گوساله‌ها، پژوهشگاه زیست‌پزشکی
بررسی سروالوژی و ملکولی لیشمایوز اشتباهی در سگ‌های ولگرد در شهرستان تربت حیدریه

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آگاه و یکی از بیماری‌های مبتنی بر عنوان انفلونزا لیشمایوز است که در ایران شناخته می‌شود. در این مطالعه با استفاده از مدل DNA و آزمایش PCR تغییرات مولکولی این بیماری در سگ‌های ولگرد شهرستان تربت حیدریه بحث می‌شود. این مطالعه سه گروه (A, B, C) در کل شهرستان تربت حیدریه، با بررسی سرما و پوست سگ‌های سرمشق و پاولی این بیماری در سایه‌های تربت حیدریه بحث می‌شود.

چکیده

لیشمایوز اشتباهی یک بیماری مهم مشترک بین انسان و دام در ایران است که عامل ناقل و مخزن آن به ترتیب لیشمایوایا اینفانتوم، فلیتوموس میجور و سگ می‌باشد. ناکامی هیچگونه اطلاعاتی درباره میزان شیوع لیشمایوز اشتباهی در سگ‌های ولگرد محلی شهرستان تربت حیدریه در تحقیق فعلی مطالعه شده است. در این مطالعه با استفاده از آزمایش PCR تغییرات مولکولی پاتوبیولوژی بیماری لیشمایوز اشتباهی در سگ‌های ولگرد محلی شهرستان تربت حیدریه بررسی شد.

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Abstracts (In Persian)
بررسی ارتباطین امتیازات بهداشتی گاوشیری با عفونتهای داخل پستانی

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چکیده

به منظور بررسی ارتباطی از امتیازات بهداشتی شامل CMT و SCC با عفونت‌های داخل پستانی در گاو شیری در یک گاوگرد گازال، 128 گاو به صورت تصادفی منسق به 4 گروه تقسیم شدند. در گروه نمونه (G1) از امیرالاکی، مبتلا به عفونت سیستم‌های داخل پستانی بودند و در گروه کنترل (G2) این عفونت رخ نداشت. نتایج نشان داد که ارتباطی به‌طور معناداری بین امتیازات بهداشتی و عفونت‌های داخل پستانی وجود نداشت.

در نتیجه، ارتباطی معناداری بین عفونت‌های داخل پستانی و سایر عوامل بهداشتی در گاو شیری در یک گاوگرد گازال وجود نداشت.

واژگان کلیدی: اسکور بهداشتی، عفونت داخل پستانی، گاو، عفونت داخل پستانی، CMT و SCC
چکیده

با توجه به گزارش‌های مبتدی در مورد طاعون نشخوارکندگان کوچک (PPR) در مناطق مختلف ایران، پرداختن در موضوع تحقیقات گوناگون بیشتری در مورد جداسازی و بررسی خصوصیات زیستی ویروس‌های موجب جدایی بیشتر این بیماری تأکید شد. واکسن بهبودی‌یابی دارد. در طی سال‌های 1393-1394 تعداد 188 نمونه خون کامل، سوابق جنگلی و کمیتی، گونه و دهانی و غدد لنفاوی (گوسفندها و بلندایاها) با علامت‌های عفونیت در ته بهشت (قم، فارس، اردبیل، کرمان، آذربایجان غربی، اصفهان، کرمان و هرمزگان)، در کنار آنان در روستاهای آذربایجان شرقی، آذربایجان غربی، اصفهان، کرمان و هرمزگان از کانون‌های آلودگی ایران جمع آوری شد. نمونه‌ها پس از آماده‌سازی، بر روی سلول Vero و سلول خانه پاریزی گلی بر (LK) تلفیق و جدید مشاهده‌ها شایعات سلولی (CPE) در مورد سلول مورد تهیه شد. در نمونه‌های گوسفندها و نمونه‌های از پوست (آذربایجان شرقی و کرمان) در هر دو نوع سلول مشاهده شد. در کشت‌های سلولی وارد، مشاهده شد که میکروسلوکیوپاکت‌های CPE مشاهده شد. سلول‌های مبتلا به طاعون NIAH در مایع‌کننده‌های RT-PCR مثبت بودند. با این نتایج، پرداختن در بخش از کانون‌های مورد مطالعه تأیید می‌گردد.

واژگان کلیدی: طاعون نشخوارکندگان کوچک، جداسازی، تشخیص، ایران
ارزیابی هیستومورفومتری و اولتراسونوگرافی دیواره شکم‌های گوسفند

محمدرضا رضا راجی، علی میر Шاهی، رضا رضا، محمد مونافی شاه، دکتر آمین شیرآبادی و رضا احمد آذری شاهی

چکیده

بروز ضایعات در دیواره شکم گوسفند می‌تواند باعث کاهش تولید و بارداری جنین شود و گاهی اوقات مراکز جنین را به همراه داشته باشد. اولتراسونوگرافی به عنوان یک روش تشخیصی بی‌خطر و درد‌رسی می‌تواند در جهت تشخیص این ضایعات در دستگاه معدی روده‌ای استفاده شود. در مطالعه حاضر، عده‌ای از خانم‌ها و شوهران سالم از کل کشور گروه گوسفند سالمند از کل کشور گروه گوسفند سالمند شدند. سپس، به آزمایشگاه گوش و گوارش دام‌پزشکی منتقل شدند. در این مطالعه از دیواره شکم‌های مرغ در نهایت به کمک آزمایشگاه گوش و گوارش، پیش‌زمینه و پیلار تغییر به گوگرد 10% (میلی‌متری) نمونه‌ها در سرخ‌پزشکی تشخیصی جهت تشخیص این ضایعات در دیواره شکم و بلعک یا دیگر روش‌های آزمایشگاهی استفاده شد. نتایج نشان‌دهنده آن است که اولتراسونوگرام‌های سالم از دیواره شکم‌های گوسفند به علت آبگیری و ثابت شدن، پیش‌بینی‌های واقعی می‌باشد و بخصوص در اولین فاصله‌های شکم‌های مرغ، استفاده از این روش در تشخیص این ضایعات می‌تواند مفید باشد.

واژگان کلیدی: اولتراسونوگرافی، هیستومورفومتری، شکم‌های گوسفند
Abstracts (In Persian)

DOI: 10.22067/veterinary.v9i1.61068

بررسی هیستومورفوزن پانکراس چنین شترمغ

م Compound اسمیان، روزه سعادتفر، حسینه قدیری آزادی

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دریافت مقاله: 1395/09/25

پذیرش نهایی: 1395/12/18

چکیده

مطالعه حاضر به منظور بررسی تکامل پانکراس در شتر مرغ انجام شد. این مطالعه بر روی 24 عدد نخ شترمرغ در سنین 20،22،24،26 و 30 روزه جنین و 1 روزگار بعد از خروج از نمک انجم شد. نتایج آزمایش های همانتوسکوپی- اتوسیم، گوموری و تولویند بلو برای بررسی نمونه ها با میکروسکوپ نوری انجام شد. در 16 روزگر، پانکراس در مجاورت کبد و روده (دو روزها) قابل مشاهده بود. در این سن پانکراس نهایا یک لوب تشکیل شده و حاوی سلول های پوشاکی نکامل نیافته بافت همبند، مجاری و خروق خونی سازمان پانکراس نیافته بودند. در 16 روزگر، پانکراس حاوی دو لوب پشتی و شکمی و از نظر ساختار بافتی مشابه 16 روزگر بود. در 16 روزگر گلاژول های زیموزن درون بخش پر و ریز مشخص شدند. با افزایش سن مرغ بین پانکراس مشخص شدند.

واژگان کلیدی: شترمرغ، بافت پانکراس، تکامل
چکیده
هدف از این مطالعه بررسی اثر جنس برای ساختارهای بافت شناسی و هیستوشیمیایی سینوس بین انگشت بیفته در گوسفندهای بالغ بیتکاری ایران بهزودی بین. هاد رنجکش و حیدر مبنی، ایران شهروند بیانی. دانشکده دامپزشکی، دانشگاه آزاد اسلامی واحد شهرکرد، شهرکرد ایران

دریافت مقاله: ۱۳۹۵/۰۷/۲۶ پذیرش نهایی: ۱۳۹۶/۰۱/۱۹

واژگان کلیدی: هیستوشیمیایی، بافت شناسی، سینوس بین انگشت، جنس گوسفندهای بالغ
Abstracts (In Persian)

DOI: 10.22067/veterinary.v9i1.53864

بررسی تأثیر نیامین و ویتامین C در کاهش تجمع بافتنی سرب به دنیال مسمومیت تجربی با سرب
در کیور معمولی

دارو شاهسونی1، حسن باغشونی2، کیمی نوریان3

گروهی که به داروهای غذایی و آبیاری، داشته، دامداری و دانشگاه فردوسی مشهد، مشهد، ایران
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دریافت مقاله: 12/12/1394    پذیرش نهایی: 1395/12/15

چکیده

مطالعه حاضر به منظور ارزیابی اثر احتمالی ویتامین C و نیامین بر کاهش تجمع سرب در کلیه کییک، عضله، مغز و آبشش ماهیان کیور معمولی در مسمومیت تجربی با سرب انجام شده است. 120 ماهی بطور تصادفی به چهار گروه تقسیم شدند. گروه 1 به عوامل گروه شاهد در نظر گرفته شد. گروه 2،3 آستات سرب (5 میلی گرم در لیتر، 15 زوم در دیرواته نمود. گروه 4 به ترتیب ویتامین C (500 میلی گرم در هر کیلوگرم غذا) و نیامین (5 میلی گرم در هر کیلوگرم غذا) در سه ماه زمان مواجهه به سرب (5 میلی گرم در لیتر، 15 زوم) دریافت نمودند. مقایسه با سرب باعث افزایش معنی دار (0.05≤P<0.01) میزان سرب در تمام بایت های مورد آزمایش ماهیان گروه 2 در مقایسه با گروه شاهد شد. نیامین، مقداری بالای سرب در بایت های عضله، مغز و آبشش را به مقابیری کاهش داد که نسبت به گروه کنترل اختلاف معنی داری نداشتند. همچنین، ویتامین C غلطی های افزایش فشاره سرب در عضله را به مقابیری که اختلاف معنی دار با گروه کنترل نداشتند، کاهش داد. بر اساس نتایج تحقیق حاضر نیامین و ویتامین C ناتانرمعنیداری در کاهش تجمع سرب در بیشتری ماهیان گروه های 3 و 4 در مقایسه با گروه 2 نداشتند. بنابراین استفاده از این ویتامین ها به نهایی به عنوان یک گردن درمانی مناسب جهت کاهش میزان سرب در کیور معمولی نمی تواند بیشتری گردد. یا این وجود بررسی های بیشتر با استفاده از دوره‌های مختلف دیگر با درمان ترکیبی با استفاده هم‌زمان از شلال‌ها ممکن است قابل

از این نتیجه گرفت واکنش کلیدی سرب باعث کاهش معمولی، نیامین، ویتامین C

کوری معمولی، نیامین، ویتامین C

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چکیده
نتیجه‌گیری‌ها در ماهیان نسبت به سایر جویان کمتر به صورت تهاجمی بوده و بیشتر به صورت مجزا و کانونی رخ می‌دهند. اگرچه تغییراتی با مشابه بیماری‌ای در ماهیان دیده می‌شود، اما فیروس‌ارکوم پوستی به دنبال تاریکی‌پوشانی (Carassius auratus) شده است. یک ماهی حوض (عکس) دچار سلول‌های دوکی شکل تکنیکی اثر در هم روده و اورینفیلیا در تکامل کم به دست آمده و در پی تشخیص افتراقی، رنگ‌آمیزی می‌شود که از دیدگاه احتمال کم‌کاری کرده و پس از انجام چند گزارش شدید علائم شکل داده شد. این اولین گزارش از خصوصیات آسیب‌شناسی بالینی فیروس‌ارکوم پوستی ماهی حوض در ایران است.

واژگان کلیدی: فیروس‌ارکوم، ماهی حوض
گزارش هیستوپاتولوژی آلودگی توسط سنتروستوس فورموسانوس در کبور علفخوار و کبور

معمولی ایران

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چکیده

ترمیت‌دها منجر به بیماری‌های شدید و کشنده در حیوانات مختلف از جمله ماهیان شده و در هر دو مراحل بالغ و نوزادی در

ماهیان پایت می‌شود. سنتروستوسیس یک عفونت مربوط به آبشین بوده که توسط سنتروستوس فورموسانوس (C. formosanus) ایجاد می‌شود. در تابستان سال 1393 ماهی کبور علفخوار و 30 کبور معمولی وکشته گردید که با وزن 6 کیلوگرم به

بیمارستان دامپزشکی دانشگاه شهید چمران اهواز به‌عنوان آبزیان ارجاع داده شد. در گسترش مرطوب به شده از

پوست، آلودگی خفیف‌تری را تیزکرده می‌نماید. گسترش مرطوب به‌انگل کیستی

این رشت‌های آبزی‌ها را نشان داد. کیست‌ها کاملاً شفاف بود و انگل کابلی شکلی درون هر کیست مشاهده شد. برسی

هیستوپاتولوژی، ضخیم شدن، خمیده شدن رشت‌های آبزی‌ها و مقاطع انگل‌ها را نشان داد. بر اساس نتایج حاصل از گسترش

مرطوب و هیستوپاتولوژی، مناسب‌کردن مشاهده شده روابط به خانواده هیستوفیده و سنتروستوس فورموسانوس بوده که منجر به

مرگ و میر بالایی ناشی از آلودگی انگل‌ی شده بود.

واژگان کلیدی: سنتروستوس فورموسانوس، کبور علفخوار، کبور معمولی، هیستوپاتولوژی
List of reviewers

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The Editor-in-Chief would like to extend his sincere gratitude to the following reviewers for evaluating and assessing manuscripts published in this issue of IJVST journal.

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Guide for Authors

Scope

Iranian journal of Veterinary Science and Technology (IJVST) is a peer-reviewed and multi-disciplinary journal that supports important research advances in veterinary medicine and subject areas relevant to veterinary medicine including anatomy, physiology, pharmacology, bacteriology, biochemistry, biotechnology, food hygiene, public health, immunology, molecular biology, parasitology, pathology, virology, etc. Contributions related to clinical sciences including large and small animal medicine, poultry disease, diseases of equine species and aquaculture are welcomed. Articles can comprise research in basic sciences, as well as applied veterinary findings and experimental studies with impacts on diagnosis, treatment and prevention of animal diseases.

General guidelines

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4. Full-length articles, Short Communications, Case reports, Reviews and Commentaries that address scope of the journal will be considered for publication.
5. Ethics: Authors must state that the protocol for the research project has been approved by the Ethics Committee of the institution within which the work was undertaken. Authors are responsible for animal welfare and all statements made in their work.

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Review Articles should provide an update on recent advances in a particular field. Authors wishing to submit review articles should contact the Editor with an outline of the proposed paper prior to submission.

Case Reports should include Title Page (including Acknowledgements, Author Contributions, and Conflict of Interest Statements), Abstract, Keywords, List of Abbreviations, Introduction, Case Presentation, Results and Discussion, and References. Case reports should not exceed 2000 words (excluding the references) and should include no more than two tables or figures.

Short Communications should not exceed 2000 words (excluding the references) and include no more than two tables or figures. They should include Title Page (including Acknowledgements, Author Contributions, and Conflict of Interest Statements), Abstract, Keywords, List of Abbreviations, the text summarizing results with no other divisions, and References.
Guide for Authors

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Introduction should be as concise as possible, and clearly explain the main objective and hypothesis of the investigation.

RESULTS

Results indicate the results of an original research in a clear and logical sequence. Do not repeat data that are already covered in tables and illustrations. In manuscripts describing more than one animal, all animals should be assigned a case number.

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Discussion should include the answer to the question proposed in the introduction and emphasize the new and important aspects of the study and the conclusions that follow from them. It could include the implication, application, or speculation of the findings and their limitations, relate the observations to other relevant studies, and links the conclusions with the goals of the study. Recommendations, when appropriate, may be included.

MATERIALS AND METHODS

Materials and Methods should be described in sufficient details to allow other workers to reproduce the results. Specify any statistical computer programs used. The methods of data collection and use of statistical analysis will be checked by the referees and if necessary, a statistician. Drugs and therapeutic agents, reagents, softwares and equipments should be given in the format: name (trade name, manufacturer name, city, country), e.g. Statview 5 (SAS Institute, Inc., Cary, NC, USA).

ACKNOWLEDGEMENTS

Personal acknowledgement, sources of financial support, contributions and helps of other researchers and everything that does not justify authorship should be mentioned in this section, if required.

AUTHOR CONTRIBUTIONS STATEMENT

Authors are required to include a statement to specify the contributions of each author. The statement describes the tasks of individual authors...
referred to by their initials. Listed below is an example of author contributions statement.

Conceived and designed the experiments: HD, SS. Performed the experiments: SS. Analyzed the data: HD, SS, MMM, ARB. Research space and equipment: HD, MMM, ARB. Contributed reagents/materials/analysis tools: HD. wrote the paper: SS, HD.

**Conflict of interest statement**

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. If there are no conflicts of interest then please state this: ‘Conflicts of interest: none’. This form can be downloaded from IJVST website.

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Example piece of text and reference list:

An unhealthy diet, obesity and physical inactivity play a role in the onset of type 2 diabetes, but it has been shown that increased physical activity substantially reduces the risk [1], and participation in regular physical activity is one of the major recommendations of the evidence based guidelines for the primary prevention of diseases [2]. According to the 2004-05 National Health Survey, more than half a million Australians (3.5% of the population) have diabetes mellitus which had been medically diagnosed and most of these people have the Type 2 condition [3]. Gestational diabetes is also on the increase, rising steadily between 2000-01 and 2005-06 [4]. Approximately two thirds of those with diabetes have been prescribed medication [3], but it is of concern that a recent review of the literature found that many people do not take their medication as prescribed [5]. Many patients also self monitor the disease by measuring their blood glucose levels with a glucose meter but Song and Lipman [6] have concerns about how well this is managed.

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