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Investigation of immunoglobulin (IgE, IgA, IgG, IgM) concentrations in calves naturally infected with coccidiosis

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Nazan Eğlenti[1], Süleyman Kozat[*1], Vural Denizhan[2]

1. Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Internal Medicine, Zeve Campus, 65080 Van-Turkey. **2.** Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Parasitology, Zeve Campus, 65080 Van-Turkey.

Eğlenti, N.: ORCID: 0000-0001-5795-4188; Kozat, Z.: ORCID: 0000-0001-5089-2623, Denizhan, V. : ORCID: 0000-0002-0531-9550

ABSTRACT

The aim of this study was to determine immunoglobulin (IgA), immunoglobulin (IgE), immunoglobulin (IgG) and immunoglobulin (IgM) levels of calves naturally infected with coccidiosis and to determine the relationship between infection and calf immunity. The material of this study consisted of 30 calves (21-44 days old) which were brought to clinics of Van Yuzuncu Yil University, Faculty of Veterinary Medicine, of which 20 calves that were diagnosed as coccidiosis by detecting *Eimeria spp*. oocysts according to native faecal examination and 10 healthy calves as control group. For hematological and biochemical analysis, blood samples were taken from all animals from the v. jugularis before treatment. According to statistical analyses; there was a statistically significant increase in hematological parameters such as white blood cell count (WBC), hematocrit value (Hct), mean corpuscular volume (MCV), monocyte count (Mon) ($P<0.05$) and neutrophil (Neu) percentage ($p<0.01$), whereas there was a statistical decrease in lymphocyte (Lym) percentage and mean corpuscular hemoglobin concentration (MCHC) ($P<0.05$) levels. Besides, there was a significant increase in urea and creatinine ($P <0.05$) levels of calves with coccidiosis compared to control group. IgA, IgE, IgG and IgM concentrations of calves with coccidiosis were significantly lower than the control group ($P <0.001$). As a result; there was a relationship between calf immunity and the risk of infection of calves with coccidiosis. It was concluded that determining passive transfer failure levels and oral immunoglobulin supplementation to calves with passive transfer failure would reduce the risk of coccidiosis in newborn calves.

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Introduction

Coccidiosis is an important disease affecting poultry, cattle, sheep, goats (Aydin and Aslan, 2012; Ocal, 2016), pigs and rabbits (Baydar and Ozubek, 2012; Sultana et al., 2014). In addition, although coccidiosis is less commonly diagnosed in dogs, cats and horses, the disease can also be observed in these animals clinically. It is especially severe in calves (Mundt et al., 2005; Baydar and Ozubek, 2012). Coccidiosis is an infectious, acute or chronic protozoan disease characterized by enteritis (Aydın and Aslan, 2012), causing poor economic losses, susceptibility to other diseases and death, especially in breeding livestock and calf breeding (Mundt et al., 2005; Cicek et al., 2007; Baydar and Ozubek, 2012). The disease causes anemia, weakness, developmental retardation and decreased productivity in animals (Mundt et al., 2005; Cicek et al., 2007;

*Corresponding Author: Süleyman Kozat
E-mail: skozat@hotmail.com

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Baydar and Ozubek, 2012). Although coccidiosis infection occurs in all seasons of the year, it occurs mostly in winter and early spring and causes severe disease especially in young calves up to 6 months (Bangoura et al., 2007; Baydar and Ozubek, 2012). As a result of studies carried out in the world, 17 Eimeria and 2 Isospora species have been reported to be the cause of cattle coccidiosis (Cicek et al., 2007; Baydar and Ozubek 2012; Bozdag, 2018). *Eimeria bovis* (E. bovis) and *Eimeria zuernii* (*E. zuernii*) are the most pathogenic species in cattle (Cicek et al., 2007; Baydar and Ozubek, 2012). In Turkey, 11 Eimeria and 1 Isospora species have been reported to cause bovine coccidiosis. Pathologically, the destruction of intestinal epithelial cells leads to the formation of villi loss, impaired absorption, enteritis, dehydration and diarrhea (Arslan et al., 2015). Various factors such as secondary diseases that cause weakening of the immune system of animals at the outbreak of the disease, weaning, excessive watery feeds such as feed change, transport, shearing, silage, maintenance, feeding, air exchange, crowded stables and lack of stable hygiene play an important role (Aydin and Aslan, 2012). As with most parasitic diseases, coccidiosis also changes many of the biochemical and hematological parameters (Arslan et al., 2015). Immunoglobulins are molecules in the immune system that have antigen-binding site and can combine with antigens that cause them to form. Thanks to these properties, they cause reactions in the body (Yılmaz and Akgul, 2014).

The main purpose of immunoglobulins; it is to be inactive by binding to the antigen and then forming the antigen-antibody compound, making it easier for the antigen to gather together and to be removed by phagocytosis (Senturk and Esen, 2012).

In this study; it was aimed to determine the immunoglobulin levels in coccidiosis disease. It is foreseen to reveal the importance of immunoglobulins in calf coccidiosis infection, which is common in veterinary medicine and causes significant economic losses.

Material and methods

The material of this study diarrhea brought to Van Yuzuncu Yil University Veterinary Faculty Clinic. This research was approved by Van Yuzuncu Yil University Animal Research Ethics Committee (07/03/2019 and Decision no: 2019/02).

Stool samples were taken from calves showing signs of diarrhea or bloody diarrhea. Blood samples were taken from V. jugularis to hematological and serum tubes in accordance with the technique for the analysis of hematological and biochemical parameters in Emeria spp. calves with oocyst detected. However, anticoccidial therapy was applied to determine whether diarrhea of blood samples taken from coccidial origin or non-coccidial origin. 20 simmental breeds, which were recorded and responded to anticoccidial therapy, and diarrhea calves with 21-44 days of age and 10 healthy calves were used. The diarrhea of calves that did not respond to anticoccidial therapy was not included in the study considering that diarrhea was caused by mixed infections. Calves with oocyst on microscopic stool examination according to clinical findings and presence of 4000 or above oocysts per gram faeces clinical findings were named as group infected with coccidiosis and healthy calves.

Calves used as subjects were subjected to a general clinical examination and their body temperature, respiratory and pulse numbers were determined. In addition, whether the calves have had any diseases in the past, whether any treatment has been applied or not has been determined by the anamnesis information received from the patient

Examination of stool samples: In the examination of stool samples; in the calves with suspected coccidiosis, 30-50 g stool sample was taken from the rectum into sterile plastic containers. Fecal samples were examined by native examination method to find Eimeria spp. In the microscopic examination of the feces, blood samples were taken from diarrhea calves with oocyst. The number of oocysts per gram of faeces was determined by the modified McMaster technique. Clinic coccidiosis was confirmed based on presence of 4000 or above oocysts per gram faeces (Chhabra and Pandey 1991; Ocal et al., 2007).

Hematological examinations: For hematological leukocyte (WBC), erythrocyte (RBC), hematocrit (Hct), lymphocyte (Lym), monocyte (Mon), neutrophil (Neu), eosinophil (Eo), basophil (Ba), mean erythrocyte volume (MCV), mean hemoglobin (MCH), mean erythrocyte hemoglobin concentration (MCHC), hemoglobin (Hb) and platelet (PLT) parameters were measured with a Veterinary Hemogram device.

Biochemical examinations: Serum samples obtained were stored in serum storage tubes at -20° C until biochemical analysis. To determine the serum immunoglobulin (IgA), immunoglobulin (IgE), immunoglobulin (IgG) and immunoglobulin (IgM) concentrations from the serum obtained, measurements were made with the ELISA device using commercial immunoglobulin test kit. Serum total protein (TP), albumin, globulin, urea and creatinine levels of calves with healthy (control) and coccidiosis were measured with the autoanalyzer device.

Calves with diarrhea and coccidiosis were treated with drugs such as Sulfadimidine sodium 16%, Trimethoprim + sulfadoxine combination as well as anticoccidial treatment, as well as calcium and vitamin C, vitamin K and epithelial destruction against severe bleeding.

Statistical analysis: Data related to the research results are presented as mean and standard error (Mean ± SD). In terms of hematological and biochemical parameter analyzes, independent t test (Independent two sample t-test) was used to compare groups with control and coccidiosis. Pearson correlation was used to determine the relationship levels between the variables. R and SAS statistical software program was used for necessary statistical analysis

Results

Clinical findings: We have been informed about the anamnesis information of the calves brought to our clinic that the calves are poor, sluggish, stagnant, painful, some have bloody stools and some calves die. As a result of the clinical examination we performed, clinical findings such as calves sluggish, stagnant, mixed hair, abdominal slanted, dehydrated, perineum region and hind limbs were contaminated with feces, increase in tenesmus and heart rate. In addition to the clinical findings detected, images of microscopic examinations of the feces of calves with coccidiosis are given in Figure 1 and Figure 2.

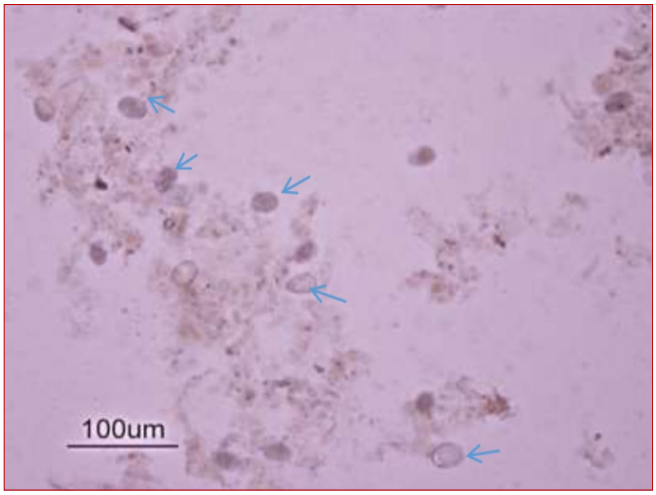

Figure 1. Microscopic image of *Eimeria spp* oocysts.

Hematological findings: Hematological parameters of coccidiosis and control groups are given in Table 1. In the optional analysis made; WBC, Hct, Mon, Neu (%) and MCV values of coccidiosis calves were significantly higher than the same parameters of the control group ($P < 0.01$, $P < 0.05$), Lymphocyte (Lym) and mean erythrocyte hemoglobin concentration (MCHC) values were found to be significantly low ($P < 0.05$) (Table 1).

Figure 2. Microscopic image of *Eimeria spp* oocysts.

Table 1. Hematological parameters in healthy and calves infected with coccidiosis

Parameters	Control group (n=10) $\bar{x} \pm SD$	Calves infected with coccidiosis (n=20) $\bar{x} \pm SD$
WBC (10^3/mm³)	10.17 ± 0.81	14.64 ± 1.31*
RBC(10^6/mm³)	8.62 ± 0.28	8.75 ± 0.44
Hct (%)	23.20 ± 2.71	28.93 ± 1.72*
Lym (10^3/mm³)	5.98 ± 0.49	6.64 ± 0.91
Mon (10^3/mm³)	0.45 ± 0.04	0.64 ± 0.06*
Neu (10^3/mm³)	3.48 ± 0.37	5.56 ± 0.39
Eos (10^3/mm³)	0.31 ± 0.05	0.59 ± 0.12
Lym (%)	59.17 ± 2.27	47.39 ± 3.20*
Mon (%)	4.59 ± 0.34	4.84 ± 0.4
Neu (%)	33.99 ± 2.35	40.42 ± 3.48**
Eos (%)	3.15 ± 0.48	3.26 ± 0.55
Bas (%)	2.96 ± 0.58	3.65 ± 0.57
MCV (fl)	26.34 ± 2.75	31.19 ± 0.68*
MCH (pg)	12.63 ± 0.38	12.83 ± 0.28
MCHC (g/dl)	43.36 ± 0.41	40.42 ± 1.01*
Hb (g/dl)	10.96 ± 0.54	11.93 ± 0.64
PLT (10^3/mm³)	629.10 ± 85.91	514.90 ± 68.66

$\bar{x} \pm SD$: mean ± standard deviation. *: $P < 0.05$, **: $P < 0.01$

Biochemical findings: The changes in the biochemical parameters of the coccidiosis and control groups are given in Table 2. As a result of statistical analysis; Urea and creatinine values of calves with coccidiosis were significantly higher than the control group ($p<0.05$). Although total protein (TP), albumin (Alb) and globulin concentrations of the patient calves were lower than the control group, they were not statistically significant (Table 2).

Serum immunoglobulin concentrations of coccidiosis and healthy calves are given in Table 3. In the statistical analysis; IgA, IgE, IgG and IgM values of calves infected with coccidiosis were significantly lower compared to the same parameters of the control group (P < 0.001) (Table 3).

Table 2. Biochemical findings in healthy and calves infected with coccidiosis.

Parameters	Control group (n=10), x̄ ± SD	Calves infected with coccidiosis (n=20), x̄ ± SD
TP (g/dl)	6.13 ± 0.41	5.54 ± 0.25
Albumin (g/dl)	2.91 ± 0.12	2.66 ± 0.11
Globulin (g/dl)	3.23 ± 0.34	2.88 ± 0.24
Urea (mg/dl)	16.84 ± 2.41	42.82 ± 8.04 *
Creatinin (mg/dl)	0.85 ± 0.09	1.38 ± 0.17 *

x̄ ± SD : mean ± standard deviation. *: P < 0.05

Table 3. Immunoglobulin concentrations in healthy and calves infected with coccidiosis.

Parameters	Control group (n=10), x̄ ± SD	Calves infected with coccidiosis (n=20), x̄ ± SD
Ig A (µg/mL)	2.73 ± 0.60	0.96 ± 0.05 ***
Ig E (ng/mL)	306.68 ± 24.44	205.62 ± 3.79 ***
Ig G (µg/mL)	8.21 ± 1.27	3.68 ± 0.17 ***
Ig M (ng/mL)	29.98 ± 2.15	17.37 ± 0.59 ***

x̄ ± SD : mean ± standard deviation. ***: P < 0.001.

Discussion

In studies conducted, it is reported that clinical coccidiosis is more common in animals younger than one year and there is a negative correlation between the rate of infection of cattle and age (Reddy et al., 2015). In another study, although coccidiosis infection occurs at all ages in calves, it is reported that it is more common in calves of 3 weeks and 6 months and that the disease occurs in the first month after birth due to an incubation period of 17 to 21 days (Goz et al., 2006). It was determined that the calves in this study were between the ages of 21-44 days and Eimeria infection was effective in the specified age group. It supports the findings of the researchers (Goz et al., 2006; Reddy et al., 2015) who stated that the disease is common in young people and causes severe clinical findings.

It has been reported that foul-smelling watery diarrhea, dehydration and obvious anemia are detected in cattle infected with *Eimeria bovis (*Reddy et al., 2015). In another study, besides fibrin intestinal tissue, abdominal pain, fever, tenesmus, weakness, loss of appetite and weight loss symptoms, clinical coccidiosis is bloody diarrhea (Koutny et al., 2012). The clinical findings obtained in this study are similar to the clinical findings that researchers (Koutny et al., 2012; Reddy et al., 2015) have stated regarding coccidiosis infection.

Many researchers state that WBC numbers are either within normal limits or below normal limits in calf coccidiosis infections (Adams et al., 1992; Knowles et al., 2000). The observed leukopenia is interpreted that the leukocyte curve is caused by the depression phase during acute infection. However, it has been reported that leukocytosis was detected in the study (Willuhn, 1999) related to bovine coccidiosis. In another study (Ghanem and Abd El-Raof, 2005), a significant level of eosinophilia was detected with leukocytosis, and these changes are assumed to be due to intestinal inflammation. In this study, leukocyte values of calves infected with coccidiosis were found higher than healthy calves. WBC values obtained from calves infected with coccidiosis were found to be in agreement with the researchers' reports (Willuhn, 1999; Ghanem and Abd El-Raof, 2005) while there was no significant difference in eosinophil value between calves with coccidiosis and healthy calves. They reported that detected lymphopenia with an increase in neutrophils in buffalo offspring infected with *Eimeria spp*. (Anwar et al., 1999). It has been shown that *Eimeria* parasites can be seen as a cellular response to intestinal invasion, as neutrophil increase in the blood and decrease in circulating lymphocytes (Rakhshandehroo et al., 2013). In the current study, it was found that the lymphocyte values of calves infected with coccidiosis showed a statistically significant decrease compared to the calves in the control group. Lymphocyte-related changes in calves with coccidiosis support the data of researchers (Anwar et al., 1999; Rakhshandehroo et al., 2013). In contrast, the neutrophil value of calves with coccidiosis was significantly higher than the control group. It is thought that the increase in neutrophil levels may be related to the cellular response to infections.

It is reported that in cattle with coccidiosis, Hb concentration is increased and Hct value is low (Knowles et al., 2000; Bangoura and Daugschies, 2007). In another study, it has been reported that Hct value decreases as a result of blood loss or anemia

that occurs in gastrointestinal system infections of newborn and adult ruminants (Heller and Chiwerge, 2018). It is reported that there are differences in Hb and Hct values between the calves and control calves (Bangoura and Daugschies, 2007). In this study, the findings we obtained regarding the increase of Hb value in calves infected with coccidiosis were found to be parallel to the findings of the researchers (Knowles et al., 2000; Bangoura and Daugschies, 2007), but this increase was not found to be statistically significant. Contrary to the findings of the researchers (Knowles et al., 2000; Bangoura and Daugschies, 2007; Heller and Chiwerge, 2018), the Hct values of the calves with coccidiosis were found to be higher ($P<0.05$) compared to the control group. It is reported that MCV concentration is low (Bangoura and Daugschies, 2007) in experimental *Eimeria zuernii* infection in calves and MCV concentration is high in the group with severe oocyst. It is assumed that this is due to hemorrhagic diarrhea and high blood and fluid loss, and that immature reticulocytes shift from bone marrow to immature reticulocytes with high cell volume (Martin and Lumsden, 1987; Adams et al., 1992). In this study, MCV value was found to be higher in infected calves whose oocyst presence was found in stool examination compared to the control group and this data was obtained by Adams et al., (1992) and Martin and Lumsden (1987) were found to be in agreement with the findings. Researchers (Anwar et al., 1999) stated that MCHC value was significantly low ($P<0.001$) in buffalo pups infected with coccidiosis and the decrease could be due to deficiencies of hematopoietic factors. They also stated that a decrease in MCHC values indicating hyperchromic anemia may be due to low blood loss and low Hb in erythrocytes. In this study, MCHC values of calves infected with coccidiosis were found lower than the control group ($P<0.01$). MCHC values of calves infected with coccidiosis support the researcher's (Anwer et al., 1999). Bangoura et al. (2007) has been reported that the TP and Alb levels of calves infected experimentally with *E. zuernii* oocysts are lower than the control group. It has been reported that serum TP values decrease in the gastrointestinal tract infections of newborn and adult ruminants as a result of blood loss or anemia (Heller and Chiwerge, 2018). In the current study, it was found that the TP and Alb levels of the calves with coccidiosis were lower than the healthy control group. However, decreases in these values were not statistically significant. Decreases in TP and Alb values are similar to the findings of the researchers (Bangoura et al., 2007; Schneider et al., 2013; Heller and Chiwerge, 2018) although they do not have a significant value. In another study (Bangoura et al., 2007), in experimental research, calves were infected orally with 150,000 sported *E. zuernii* oocytes (moderate infection) and 250,000 sported *E. zuernii* oocytes (severe infection) per calf, respectively. While TP and Alb concentrations of moderately infected calves were significantly lower than healthy calves ($P<0.05$), TP and Alb concentrations of severely infected calves were reported to be high. The increase in serum protein levels of severely infected calves reflects increased fluid-related hemoconcentration rather than increased Hct levels and increased availability of serum protein. In general, It is assumed that diarrhea; causes intestinal protein loss and consequently a decreased TP serum concentration (Fitzgerald and Mansfield, 1973; Stockdale et al., 1981). In this study, TP and Alb findings of the calves with coccidiosis were found consistent with the findings of the researchers (Fitzgerald and Mansfield, 1973; Stockdale et al., 1981; Bangoura et al., 2007).

It has been reported that serum Hct values decrease simultaneously as a result of blood loss or anemia occurring in gastrointestinal system infections of newborn and adult ruminants (Heller and Chiwerge, 2018). In our study, although calves with coccidiosis were not considered statistically significant, a decrease in TP was detected. However, Hct value was found to be high at $P<0.05$ in contrast to the findings of the researchers (Knowles et al., 2000; Bangoura and Daugschies, 2007). The reason for this; it is believed that the damage that may cause bleeding in the intestinal mucosa due to the presence of severe dehydration and early infection in calves with diarrhea due to coccidiosis.

In another study (Aydogdu, 2014), there was a positive correlation between TP concentrations and adequate passive transfer, and the serum total protein concentration in newborn calves was > 6 g/dl, the calf had sufficient passive transfer, and the serum total protein concentration was <5 g/dl. It is reported that it can be evaluated as transfer failure. In this study, total protein was found to be 6.13 ± 0.41 gr/dl in healthy calves, while the total protein value of calves with coccidiosis was determined as 5.54 ± 0.25 gr/dl. These parameters support the researcher's (Aydogdu, 2014) passive transfer failure and total protein value findings.

Diseases and deaths of newborn calves are important causes of economic loss in the livestock sector (Kozat, 2018). Colostrum is important for the

growth and health of newborn offspring. Colostrum has a wide antimicrobial effect thanks to the antibody (immunoglobulin) complement system it contains and provides passive immunity to the calf until the immune system matures (Kozat, 2019). There are three different forms of antibodies in the colostrum, namely IgG (IgG1, IgG2), IgA and IgM. 85-90 % of total immunoglobulins are IgG, 5 % IgA and 7 % IgM (Kozat, 2019). In addition, IgD and IgE are other Ig types that are less common (Gokce and Erdogan, 2013). Calves in the first 3 to 4 weeks of life can secrete 25 to 30 % of absorbed antibodies in the gastrointestinal tract. However, non-immunized, naturally infected calves have been reported to have low antibody levels against enteropathogens (Al-Alo et al., 2018). The level of passive immune transfer in calves is not only dependent on the immunoglobulin concentration in the colostrum, but the mother's antibody production is also associated with genetics, herd management, nutritional status, and the number of lactations (Kozat, 2018; Kozat, 2019).

It has been reported that calves whose serum Ig concentration exceeding 10 g/L in the 30 to 60 hour period of their lives do not become ill before 14 days and the morbidity and disease density is very low (Furman-Fratczak et al., 2011). It confirms the data of many researches that there is a relationship between low serum Ig concentrations of neonatal calves and disease and death events and emphasizes the importance of a passive immune transfer suitable for calf health (Lora et al., 2018; Kozat, 2019). While colostral immunoglobulins provide defense in the treatment and prevention of viral and bacterial infections, growth factors promote intestinal integrity and mucosal healing of diarrhea calves through differentiation of immature and mature cells in the gastrointestinal tract (Chung et al., 2019; Kozat 2019). In the current study, the Ig A, IgE, IgG, Ig M values of the calves infected with coccidiosis were found to be significantly lower compared to the same parameters of the control group ($P<0.01$) (Table 3). In this study, decreases in immunoglobulin concentrations in calves infected with coccidiosis support data from many investigators (Furman-Fratczak et al., 2011; Al-Alo et al., 2018; Lora et al., 2018; Chung et al., 2019), which reveal relationships between infection and immunoglobulin levels.

As a result, it was revealed that there is a relationship between the risk of infection of calves with coccidiosis and calf immunity. It was concluded that determining the level of passive transfer failure to support neonatal calves to avoid coccidiosis infection and supplementing the calves with deficiency with oral immunoglobulins will reduce the risk of developing coccidial infections.

Conflict of Interest

In this study, I declare that there are no conflicts of interest among the authors.

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References

Adams, R., Garry, F. B., Aldridge, B. M., Holland, M. D., & Odde, K. G. (1992). Haematologic values in newborn beef calves. *American Journal of Veterinary Research,* 53, 944-950.

Al-Alo, K. Z. K., Brujeni, G. N., Lotfollahzadeh, S., Moosakhani, F., & Gharabaghi, A. (2018). Correlation between neonatal calf diarrhea and the level of maternally derived antibodies. *Iranian Journal of Veterinary Research,* 19, 3-8.

Anwar, A. H., Kazmi, S. I. H., & Khan, M. N. (1999). Effects of experimentally induced coccidiosis on some blood parameters of buffalo calves. *Pakistan Journal of Biological Sciences,* 2, 1024-1026.

Arslan, M. O., Kirmizigul, A. H., Parmaksizoglu, N., & Erkilic, E. E. (2015). The first case of winter coccidiosis in naturally infected calves with *Eimeria zuernii*. *Atatürk Üniversitesi Veteriner Bilimler Dergisi,* 10, 193-197.

Aydın, U. E. & Aslan, O. (2012). The determination of some coagulation parameters at calves cocciodiosis. *Veteriner Hekimler Derneği Dergisi,* 83, 1-8.

Aydogdu, U. (2014). Sütçü ineklerde kolostrum kompozisyonu ve kalitesinin buzağı pasif immunitesine etkileri. *PhD thesis,* University of Selçuk, Konya, Turkey .

Bangoura, B., & Daugschies, A. (2007a). Parasitological and clinical parameters of experimental *Eimeria zuernii* infection in calves and influence on weight gain and haemogram. *Parasitology Research,* 100, 1331-1340.

Bangoura, B., Daugschies, A., & Fuerll, M. (2007b). Influence of experimental *Eimeria zuernii* infection on clinical blood chemistry in calves. *Veterinary Parasitology,* 150, 46-53.

Baydar, E., & Ozubek, S. (2012). A Case of clinical coccidiosis in A Holstein cow. *Fırat University Veterinary Journal of Health Sciences*, 26, 111 -114.

Bozdag, A. (2018). The prevalence of Eimeria species in calves and calves in Elazig Karakocan region. *PhD thesis,* Bingol University, Institute of Science and Technology, Bingöl, Turkey.

Chhabra, R. C., & Pandey, V. S. (1991). Coccidia of goats in Zimbabwe. *Veterinary Parasitology, 39*(3-4), 199-205.

Chung, J. J., Rayburn, M. C., & Chigerwe, M. (2019). Randomized controlled clinical trial on the effect of oral immunoglobulin supplementation on neonatal dairy calves with diarrhea. *Journal of Veterinary Internal Medicine,* 33, 1807-1813.

Cicek, H., Sevimli, F., Kozan, E., Köse, M., Eser, M., & Dogan, N. (2007). Prevalence of coccidia in beef cattle in western Turkey. *Parasitology Research,* 101, 1239-1243.

Fitzgerald, P. R., & Mansfield, M. E. (1973). Efficacy of monensin against bovine coccidiosis in young Holstein Friesian calves. *The Journal of Protozoology,* 20, 121-126.

Furman-Fratczak, K., Rzasa, A., & Stefaniak, T. (2011). The influence of colostral immunoglobulin concentration in heifer calves serum on their health and growth. *Journal of Dairy Sciences,* 94, 5536-5543.

Ghanem, M. M., & El-Raof, Y. M. (2005). Clinical and haemato-biochemical studies on lamb coccidiosis and changes following amprolium and sulphadimthoxıne therapy. *Benha Veterinary Medical Journal,* 286-299.

Gokce, E., & Erdogan, H. M. (2013). Passive transfer of colostral immunoglobulins in neonatal calves. *Turkiye Klinikleri Journal of Veterinary Sciences,* 4, 18-46.

Goz, Y., Altug, N., Yuksek, N., & Ozkan, C. (2006). Parasites detected in neonatal and young calves with diarrhoea. *Bulletin of the Veterinary Institute in Pulawy,* 50, 345-348.

Heller, M. C., & Chiwerge, M. (2018). Diagnosis and treatment of infectious enteritis in neonatal and juvenile ruminants.*Veterinary Clinics of North America: Food Animal Practice,* 34, 101-117.

Knowles, T. G., Edwards, J. E., Bazeley, K. J., Brown, S. N., Butterworth, A., & Warriss, P. D. (2000). Changes in the blood biochemical and haematological profile of neonatal calves with age. *Veterinary Record,* 147, 593-598.

Koutny, H., Joachim, A., Tichy, A., & Baumgartner, W. (2012). Bovine *Eimeria* species in Austria. *Parasitology Research*, 110, 1893-1901.

Kozat, S. (2018). Hypothermia in newborn calves. *Journal of Istanbul Veterinary Sciences,* 2, 30-37.

Kozat, S. (2019). The importance of colostrum management in newborn calves *Atatürk University Journal of Veterinary Sciences,* 14, 343-353.

Lora, I., Gottardo, F., Contiero, B., Ava, B. D., Bonfanti, L., Stefani, A., & Baeberio, A. (2018). Association between passive immunity and health status of dairy calves under 30 days of age. *Preventive Veterinary Medicine,* 152, 12-15.

Martin, S. W., & Lumsden, J.H. (1987). The relationship of hematology and serum chemistry parameters to treatment for respiratory disease and weight gain in Ontario feedlot calves. *Preventive Veterinary Medicine,* 51, 499.

Mundt, H. C., Bangoura, B., Mengel, H., Keidel, J., & Daugschies, A. (2005). Control of clinical coccidiosis of calves due to Eimeria bovis and Eimeria zuernii with toltrazuril under field conditions. *Parasitology Research,* 97, 134-42.

Ocal, N. (2016). Nervöz Koksidiyozis. *Turkiye Klinikleri Veterinary Sciences-Internal Medicine-Special Topics, 2*(1), 63-67.

Ocal, N., Yagci, B. B., Duru, S. Y., & Kul, O. (2007). Toltrazuril treatment for acute clinical coccidiosis in hair goat kids: clinical pathological, haematologic and biochemical findings. *Medycyna Weterynaryjna, 63(*7), 805.

Rakhshandehroo, E., Nazifi, S., Razavi, S. M., Ghane, M., & Alavi, A. M. (2013). Caprine coccidiosis: the effects of induced infection on certain blood parameters. *Veterinarski Arhiv,* 83, 623-631.

Reddy, B. S., Sivajothi, S., & Rayulu, V. C. (2015). Clinical coccidiosis in adult cattle. *Journal of parasitic diseases,* 39, 557-559.

Schneider, A., Corrêa, M. N., & Butler, W. R. (2013). Acute phase proteins in Holstein cows diagnosed with uterine infection. *Research in Veterinary Science,* 95, 269-271.

Şentürk, E., & Esen, F. (2012). Sepsiste immünoglobulin tedavisi ile kompleman inhibisyonu ve nöroproteksiyon. *Türk Anesteziyoloji ve Reanimasyon Dernegi dergisi,* 40, 184-192.

Stockdale, P. H., Bainborough, A. R., Bailey, C. B., & Niilo, L. (1981). Some pathophysiological changes associated with infection of Eimeria zuernii in calves. *Canadian Journal of Comparative Medicine and Veterinary Science,* 45,34.

Sultana, R., Maqbool, A., Ahmad, M. U. D., Anjum, A. A., Ilyas, S. C., & Ahmad, M. S. (2014). Control of coccidiosis in calves by vaccination. *Journal of Bacteriology and Parasitology,* 5,1.

Willuhn, J. (1999). Untersuchungen der butzusammen setzung und charakterisierung peripherer blutlymphozyten im verlauf experimenteller *Eimeria bovis* infektionen des kalbes. *Fachverlag Köhler Giessen*, 98.

Yilmaz, N., & Akgul, Y. (2014). İmmünglobulinler ve septisemi. *Uludag University Journal of Faculty of Veterinary Medicine,* 33, 33-42.

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A case of feline fibroepithelial hyperplasia in a male cat

Çağla Nur Küçükbekir[1], Zeynep Günay Uçmak[2]*, İsmail Kırşan[2], Çağatay Tek[2]

1. Institute of Graduate Education, University of Istanbul- Cerrahpasa, Istanbul, Turkey. **2.** University of Istanbul- Cerrahpasa, Faculty of Veterinary Medicine, Department of Obstetrics and Gyneacology, Istanbul, Turkey

Küçükbekir, Ç. N.: ORCID: 0000-0002-9687-1702; Günay Uçmak, Z.: ORCID: 0000-0003-2530-1291, Kırşan I. : ORCID: 0000-0003-0780-0118, Tek, Ç.: ORCID: 0000-0002-6429-658X

Case Report

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ABSTRACT

A nine-month old, orange tabby, intact male cat was brought to our clinic with the complaint of swelling in the mammary glands. In anamnesis; long acting progesterone was administered to the cat in a private clinic for suppression of oestrus one week ago. As a result of clinical examination, fibroepithelial hyperplasia was detected in all mammary glands. For the purpose of treatment, aglepristone started to be used for sequential five days. Because ulceration and necrosis occurred in the mammary glands at the end of the first week, the treatment was completed with total mastectomy section. In this article, a case of fibroepithelial mammary hyperplasia which was formed as a result of progesterone administration to suppress the oestrus in a male cat is described.

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Introduction

Approximately 80% of the masses in feline mammary glands are neoplastic. The benign 20% of these masses are mammary fibroadenomatosis hyperplasia, which are commonly known as fibroepithelial hyperplasia (FEH) or feline mammary hyperplasia (Görlinger et al., 2002; Allen, 1973). This condition is characterized by non-neoplastic proliferation of interlobuler ducts and periductal stromal cells (Goldschmidt et al., 2011). Fibroepithelial hyperplasia can affect all or most of the mammary glands without covering peripheral lymph nodes and it causes enlargement on several or all of the mammary glands. Meanwhile, rapid and non-neoplastic proliferation at the ductal epithelium and stroma of the mammary gland is occurred and this is usually not accompanied by milk secretion (Allen, 1973). It is known that effect of progesterone in the body or externally applied synthetic progestins play role in its etiology. It is stated that this disease is occurred by the excessive response of the mammary glands to the physiological concentrations of endogenous progesterone or external progestin administration (Burstyn, 2010). Fibroepithelial hyperplasia is frequently seen in young, non-pregnant or pregnant female cats under the influence of luteal progesterone (Allen, 1973; Hayden et al., 1981; Johnston et al., 2001; Görlinger et al., 2002). This condition is generally seen in pubertas, in the first oestrus, during pregnancy or pseudopregnancy. Also it is commonly arise in young cats (13 weeks to 2 years old). Cases of fibroepithelial hyperplasia also occur in male and female cats of all ages with synthetic progestin (Hayden et al., 1981; Hayden et al., 1989; JohnsGörlinger et al., 2002,

*Corresponding Author: Zeynep Günay Uçmak
E-mail: vethekzeynep86@gmail.com

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Mandel, 1975). This is due to the increase in the secretion of growth hormone (GH) that produced as a side effect of the presence of ton et al., 2001; Loretti et al., 2005). However, this disease rarely arises without progesterone intake in neutered male and female cats (natural or synthetic progesterone (Mol JA et al., 1995, Mol JA et al., 1996).

Fibroepithelial hyperplasia is diagnosed as enlarged mammary glands in jelly consistency without any signs of inflammation in the initial stage. In severe cases; inflammation, tissue necrosis, ulceration, and infection may occur. The affected mammary glands of the cat with fibroepithelial hyperplasia are soft, limited and jelly-like. In addition, some patients have erythematous and necrotic areas on their skin. Anemic mucous membranes, high fever, tachycardia, apathy and anorexia can be revealed as systemic effects of the disease (Loretti et al., 2004). In the sonographic examination of the affected mammary glands, homogeneous and granular structure of parenchyma is characteristically seen. As assistant diagnostic techniques; histopathological examination of fine needle aspiration biopsy or excisional biopsy samples is also recommended (Vitasek and Dendisova, 2006; Wehrend et al., 2001).

The sexual activity of healthy male cats is manifested by intense scented urine spraying to various areas, especially when the cat is kept indoors (Dolezel et al., 2001). This behavior can be eliminated by castration. In females, progestins (eg. Medroxyprogesterone acetate, melengesterol acetate and megestrol acetate) are used in veterinary medicine to temporarily and reversibly suppress or prevent sexual activity (Romagnoli and Concannon, 2003). However, progestins applied externally in females can sometimes cause FEH in mammary glands (Hinton and Gaskell, 1977; Betlehem and van der Luer, 1993; De Souza, 2002; MacDougall, 2003; Loretti et al., 2005). In cases of feline fibroepithelial hyperplasia, regression of the enlarged mammary glands could not be possible to regress spontaneously. Treatment can only be made with the use of drugs with luteolytic effect and / or drugs to prevent endogenous progesterone effect, ovariectomy, ovariohysterectomy (Johnston et al., 2001; Keskin et al., 2008). Treatment options include total and partial mastectomy, but for medical treatment; FEH is successfully treated with the application of progesterone receptor blockers (Görlinger et al., 2002). Researchers (Görlinger et al., 2002; Nak et al., 2004; Vitasek and Dendisova, 2006; Wehrend et al., 2001) reported that they achieved successful results by applying different treatment protocols with antigestagens.

A case of mammary gland hyperplasia which developed as a side effect of progesterone administration in order to suppress sexual activity in a male cat was presented in this article.

Case

A 9 month old, weighted 4 kg, orange tabby male cat was presented to University Faculty of Veterinary Medicine Department of Obstetrics and Gynecology with swelling of the mammary glands. On anamnesis, it was reported that progesterone (Depo-Provera®, Pfizer, Belgium) was administered for once, to suppress sexual activity. On physical examination, the cat's body temperature (rectal temperature), pulse and respiratory rate were within normal limits. On inspection, it was observed that all mammary lobes (8 lobes) of the patient were excessively swollen and the skin of the mammary was stretched (Figure 1). No inflammation, ulceration and necrosis were detected in the mammary tissue. On palpation, it was determined that the mammary glands are quite jelly-like.

Complete blood count (CBC) and some biochemical parameters were evaluated in the blood samples. As a result, it was determined that reticulocyte-hemoglobin value was low (12.5 pg) and all other parameters were within normal ranges. However, levels of serum progesterone and serum testosterone were detected within normal ranges as 0.3 ng / ml and 25 ng / dl, respectively. In the ultrasonographic examination, 8 enlarged mammary lobes were visualized as encapsulated (3.3mm thick), with smooth border, hypoechogenic, and approximately 2.4x4 cm in diameter (Figure 2A-2B). Color doppler ultrasonography revealed peripheral and weak vascularization in the enlarged mammary tissues (Figure 2B).

Based on the anamnesis and typical clinical findings, the patient was diagnosed with fibroepithelial mammary hyperplasia. Since there was no necrosis and ulceration in the hyperplastic mammary glands, medical therapy was applied firstly. For this purpose; Aglepristone (Alizine, Virbac®, France) was administered once daily for 5 days at a dose of 15 mg / kg. Aglepristone has special administration way. It is applied subcutaneous way at the inner side of back leg to avoid the local alopecia and deposition of the drug into the adipose tissue.

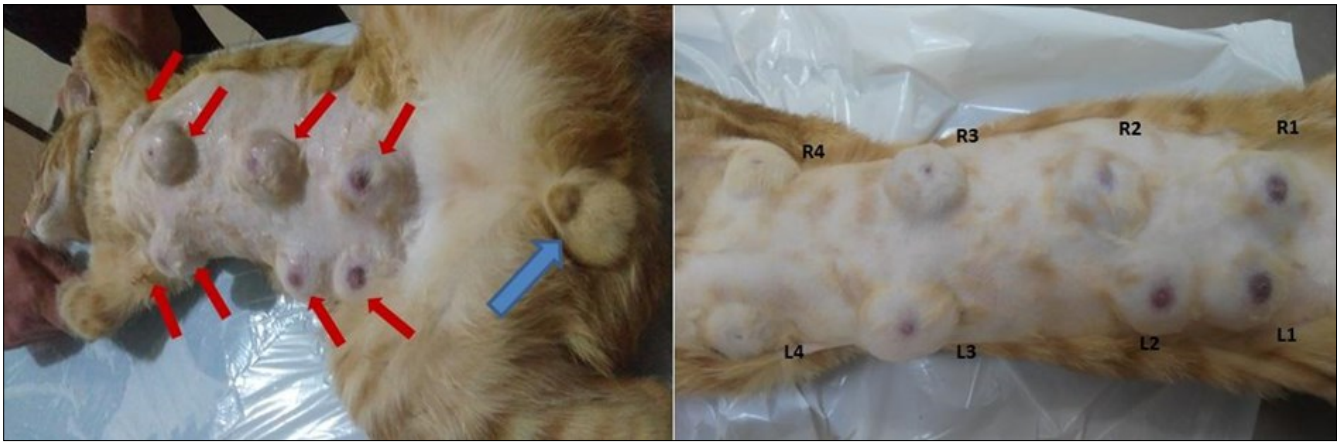

Figure 1. Enlarged mammary glands in a male cat. Red arrows: Enlarged mammary lobes, Blue arrow: Gonads of male cat. R: Right chain of mammary glands, L: Left chain of mammary glands.

Chondroitin polysulfide containing topical cream cold compresses were applied daily (Hirudoid, Santa Farma, Turkey) to the region. During the treatment, no side effects such as tachycardia and hair loss were observed. According to the control examination performed at the end of one week, there was no regression in the mammary glands and ulceration started. Therefore, the treatment was continued surgically and total mastectomy was performed to the cat. Atropine sulfate (0.03 mg / kg subcutaneously (sc); Atropin®, Teknovet, Turkey) was administrated for premedication. After induction with 1% propofol (4 mg / kg, intravenously (iv); Lipuro®, Braun, UK), general anesthesia was continued with isoflurane (% 3) (Forane Liquid®, Abbott Laboratories, UK) and oxygen (% 0.5 - % 1). Because the male cat was less than a year old (9 month), castration was not performed during the total mastectomy operation. No complications occurred after total mastectomy. After the operation, isotonic 0.9% sodium chloride (Isotonic Eczacibasi, Turkey) was administered once daily at a dose of 10 ml / kg for three days. Also the cat was treated with amoxicillin clavulanic acid (sid, 20mg/kg, sc; Synulox®, Pfizer, USA), enrofloxacin 5% (sid, 5 mg/ kg, sc; Baytril-K® 5%, Bayer, Turkey), vitamin B12 (40 mg/kg, im, sid; Dodex®, Deva, Turkey) and ranitidine (sid, 1 mg / kg, im; Ulcuran®, Yavuz Drug, Turkey) for a week. Skin sutures were removed on the fifteenth day after the operation.

Discussion

Fibroepithelial hyperplasia is defined as enlargement of one or all mammary glands without milk production (Bethlehem and Van der I., 1993). It has also been reported that FEH in female cats can be seen during pseudopregnancy and usually occurs under the influence of endogenous progesterone (Görlinger et al., 2002). However, in neutered or sexually intact

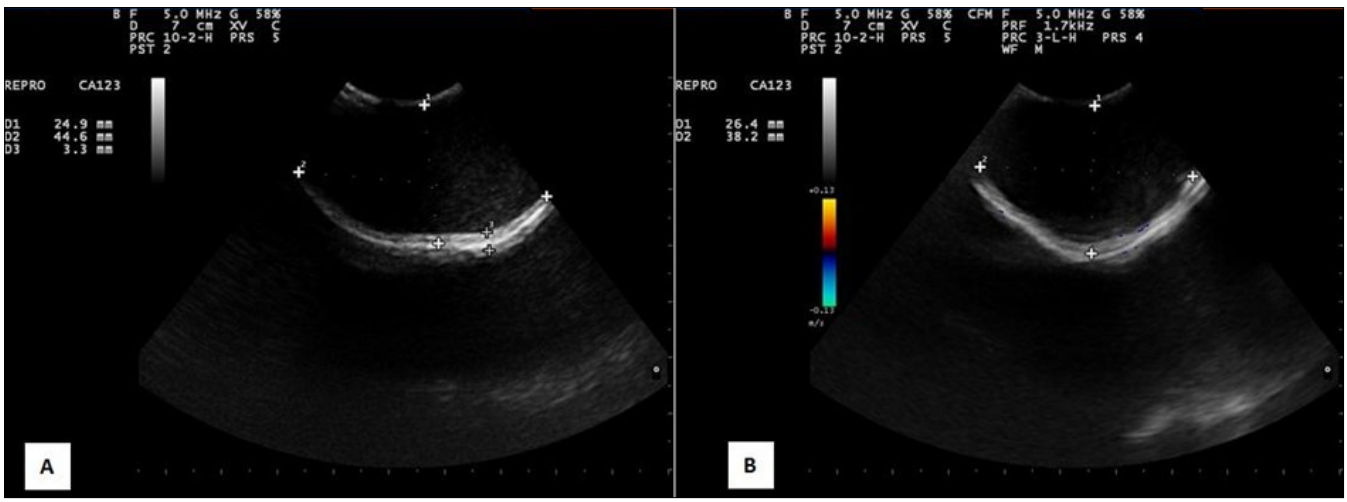

Figure 2. A: B-mod ultrasonography image and measurement of enlarged mammary lobe. B: Color doppler images and measurement of enlarged mammary lobe

female cats, FEH can occur by external administration of progesterone containing compounds (Medroxyprogesterone acetate or megestrol acetate) (Nak et al., 2004; Uçmak et al., 2011). In line with the previous reports (Nak et al., 2004; Uçmak et al., 2011), FEH was shaped immediately after long acting (3 months) progesterone administration detected FEH in 1 castrated and 2 non-castrated male cats (Görlinger et al., 2002). Similar to Görlinger et al. (2002), FEH was detected in non-castrated male cat. The researchers (Nak et al., 2004; Wehrend et al., 2001) reported that aglepristone is administered subcutaneously at a dose of 10 mg/kg for 5 times in 21 days (1-2-7-14-21th days) for the treatment of feline FEH. On the other hand Uçmak et al. (2011) achieved successful results in the treatment of FEH by using subcutaneous administration of aglepristone at the dose of 15 mg / kg for 5 consecutive days. In accordance with the researchers' report (Uçmak et al., 2011), aglepristone was administered at the dose of 15 mg/ kg subcutaneously from the inside of the back legs for 5 consecutive days for the treatment of FEH in the presented case. Görlinger et al. (2002) reported that they also treated FEH in male cats using progesterone receptor blockers. In contrast to Görlinger et al. (2002), treatment with aglepristone could not be continued for more than a week due to ulceration and necrosis in the mammary glands. Similar with Chisholm (1993), mastectomy was performed as an appropriate treatment option in

(Piyarungsri et al., 2020) reported a high risk of lower urinary tract disease (FLUTD) in male cats due to the castration performed before one year of age. In line with the previous report, castration was not performed during the surgical intervention, because the male cat was 9 months old in this case.

In conclusion, FEH could develop in mammary glands of male cats as a result of progesterone administration to with urine. Additionally; it has been concluded that surgical intervention should be considered as an altsuppress the sexual activity and its symptoms such as marking ernative treatment option in cases which do not respond to medication.

References

Allen, H. L. (1973). Feline mammary hypertrophy. *Veterinary Pathology,* 10, 501-508.

Baştan, A., Fındık, M., Erünal, N., Aslan, S., & Kılıçoğlu, Ç. (1998). The use of cabergoline for the treatment of pseudopregnancy in dogs with the purpose of suppressing lactation. *Reproduction in Domestic Animal,* 33, 49-53.

Bethlehem, M., & Van Der Luer, R. J. (1993). Feline fibroepithelial hyperplasia in 3 castrated tomcats following treatment with progestagens. *Tijdschrift Voor Diergeneeskunde,* 15, 650-652.

Burstyn, U. (2010). Management of mastitis and abscessation of mammary glands secondary to fibroadenomatous hyperplasia in a primiparturient cat. *Journal of the American Veterinary Medical Association,* 236, 326-329.

Chisholm, H. (1993). Massive mammary enlargement in a cat. *Canadian Veterinary Journal,* 34, 315.

De Souza, T. M., Fighere, R. A., Langohr, I. M., & De Bartos, C. S. L. (2002). Hiperplasia fibroepithelial mamaria em felinos: Cinco casos. *Ciencia Rural,* 32, 891-894.

Dolezel, R., Vitasek, R., & Senior, D. F. (2001). Disturbances of reproductive system. *Small Animal Veterinary Association,* 1253-1357.

Goldschmidt, M., Pena, L., Rasotto. R., & Zappulli, V. (2011). Classification and grading of canine mammary tumors. *Veterinary Pathology, 48,* 117-131.

Görlinger, S., Kooistra, H. S., Van Den Broek, A., & Okkens, A. C. (2002). Treatment of fibroadenomatous hyperplasia in cats with aglepristone. *Journal of Veterinary Internal Medicine,* 16, 710- 713.

Gültiken, N., Aslan, S., Ay, S. S., Gülbahar, M. Y., Thuróczy, J., Koldaş, E., Kaya, D, Fındık, M., & Schäfer Somi, S. (2017). Effect of deslorelin on testicular function, serum dihydrotestosterone and oestradiol concentrations during and after suppression of sexual activity in tom cats. *Journal of Feline Medicine and Surgery, 19*(2), 123-131.

Hayden, D. W., Johnston, S. D., Kiang, D. T., Johnson, K. H., & Barnes, D. M. (1981). Feline mammary hypertrophy fibroadenoma complex: clinical and hormonal aspects. *American Journal of Veterinary Research,* 42, 1699–1703.

Hayden, D. W., Barnes, D. M., & Johnson, K. H. (1989). Morphologic changes in the mammary gland

of megestrol acetatetreated and untreated cats: a retrospective study. *Veterinary Pathology,* 26, 104-113.

Hinton, M., & Gaskell, C. J. (1977). Non-neoplastic mammary hypertrophy in the cat associated ether with pregnancy or with oral progestagen therapy. *Veterinary Record,* 100, 277-280.

Jelinek, F., Barton, R., Posekana, J., & Hasonova, L. (2007). Gynaecomastia in a tom-cat caused by cyproterone acetate: a case report. *Veterinarni Medicina, 52*(11), 521–525.

Johnston, S. D., Kustritz, M. V. R., & Olson, P. N. S. (2001). *Mammary hypertropy. Canine and feline theriogenology.* Philadelphia, PA: Saunders.

Keskin, A., Yılmazbaş, G., Şimşek, G., & İntaş, K. S. (2008). Mammary hyperplasia in a pregnant queen. *Australian Veterinary Practitoner*, 38, 75.

Loretti, A. P., Ilha, M. R. S., Breitsameter, I., & Faraca, C. S. (2004). Clinical and pathological study of feline mammary fibroadenomatous change associated with depot medroxyprogesterone acetate therapy. *Arquivo Brasilero de Medicina Veterinariae Zootecnia, 56*(2), 270-274.

Loretti, A. P., Ilha, M. R., Ordas, J., & Martin de las Mulas, J. (2005). Clinical, pathological and immunohistochemical study of feline mammary fibroepithelial hyperplasia following a single injection of depot medroxyprogesterone acetate. *Journal of Feline Medicine Surgery,* 7, 43–52.

MacDougall, L. D. (2003). Mammary fibroadenomatous hyperplasia in a young cat attributed to treatment with megestrol acetate. *Canadian Veterinary Journal,* 44, 227-229.

Mandel, M. (1975). Spontaneous remission of feline benign mammary hypertrophy. *Veterinary Medicine Small Animal Clinician,* 70, 846-847.

Mayayol, S. L., Bo, S., & Pisu, M. C. (2018). Mammary fibroadenomatous hyperplasia in a male cat. *Journal* of *Feline* Medicine *and Surgery Open Reports.* 1-5.

Mol, J. A., Henzen-Logmans, S. C., Hageman, P., Misdorp, W., Blankenstein, M. A., & Rijnberk, A. (1995). Expression of the gene encoding growth hormone in the human mammary gland. *The Journal of Clinical Endocrinology and Metabolism,* 80, 3094-3096.

Mol, J. A., Van Garderen, E., Rutteman, G. R., & Rijnberk, A. (1996). New insights in the molecular mechanism of progestin-induced proliferation of mammary epithelium: induction of the local biosynthesis of growth hormone (GH) in the mammary glands of dogs, cats and humans. *The Journal of Steroid Biochemistry and Molecular Biology,* 57, 67-71.

Nak, D., Nak, Y., İntaş, K. S., & Kumru, İ. H. (2004). Treatment of feline mammary fibroadenomatous hyperplasia with aglepristone. *Australian Veterinary Practitoner, 34*(4), 161-162.

Özenç, E., & Bozkurt, M. F. (2014). Atypical fibroepithelial hyperplasia of the teats in a Sphynx cat: a case report. *Veterinární medicína,59*(5), 265-269.

Piyarungsri, K., Tangtrongsup, S., Thitaram, N., Lekklar, P., & Kittinuntasilp, A. (2020). Prevalence and risk factors of feline lower urinary tract. *Scientific Reports,* 10, 196.

Romagnoli, S., & Concannon, P. W. (2003). Clinical use of progestins in bitches and queens: a review. Recent Advances in Small Animal Reproduction. International Veterinary Information Service Ithaca, A1206.0903.

Uçmak, M., Enginler, S. Ö., Gündüz, M. C., Kırşan, İ., & Sönmez, K. (2011). Treatment of feline mammary fibroepithelial hyperplasia with the combination of aglepristone and cabergoline. *Journal of the Faculty of Veterinary Medicine, Istanbul University*, *37*(1), 69-73.

Verstegen, J. P. (1998). Pharmacological control of reproduction in the cat. *Manuel of small animal reproduction and neonatology*, 219-226.

Vitasek, R, & Dendisova, H. (2006). Treatment of feline fibroepithelial hyperplasia following a single injection of proligestone. *Acta Veterinaria Brno*, 75, 295-297.

Wehrend, A., Hospes, R., & Gruber, A.D. (2001). Treatment of feline fibroadenomatous hyperplasia with a progesterone antagonist. *Veterinary Record*, 148, 346-347.

Zertova, H. (2001). Disorders of behaviour in dogs and cats. *Czech Small Animal Veterinary Association*, 1645-1679.

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Expression of PCNA, MMP-9, p53, Bax and Bcl-2 in canine transmissible venereal tumors

Emin Karakurt[1], Mushap Kuru[2], Serpil Dağ[1], Enver Beytut[1], Hilmi Nuhoğlu[1], Murat Can Demir[2], Cihan Kaçar[2], Özhan Karataş[3] Ayfer Yıldız[4]

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1.Kafkas University, Veterinary Faculty, Department of Pathology 36100, Kars, Turkey. **2.**Kafkas University, Veterinary Faculty, Department of Obstetrics and Gynecology, 36100, Kars, Turkey. **3.**Cumhuriyet University, Veterinary Faculty, Department of Pathology, 58140, Sivas, Turkey, **4.** Kafkas University, Institute Health Sciences, 36100, Kars, Turkey.

Karakurt E.: ORCID: 0000-0003-2019-3690; Mushap K.: ORCID: 0000-0003-4409-251X, Dağ S.: ORCID: 0000-0001-7667-689X, Beytut E.: ORCID: 0000-0003-3360-2940; Nuhoğlu H.: ORCID: 0000-0003-2530-2542; Demir M.C.: ORCID: 0000-0002-3803-069X; Kaçar C.: ORCID: 0000-0002-2642-697X; Karataş Ö. ORCID:0000-0002-2778-8059 ; Yıldız A.: ORCID: 0000-0002-6569-5435 .

ABSTRACT

In this study, we aimed to evaluate the proliferative, metastatic and apoptotic capacities of TVT cases, which are in various phases of development, by using immunohistochemical markers. The material of this study consisted of twelve female and six male dogs diagnosed with TVT brought to our department between 2007 and 2020 years. Diff-quick staining was applied to the smear taken from tumoral masses for cytological examinations. Tumoral tissues from dogs were fixed in a 10% neutral buffered formaldehyde solution. After routine tissue procedures Hematoxylin & Eosin stain was applied to the sections. Tissue sections were investigated under a light microscope and photographed. Immunohistochemical staining was performed on the tissues using the avidin-biotin immune peroxidase complex method. As a result of macroscopic, cytological and histopathological examinations, TVT positive cases were divided into three according to their developmental stages. While the expression of PCNA, MMP-9, mutant p53 and Bcl-2 increased significantly in progressive cases compared to regressive and stable cases, Bax expression increased significantly in regressive cases compared to progressive and stable cases. In conclusion, we thought that the mentioned markers are very useful for understanding the prognosis of TVT, the tumor aggressiveness and the survival of the malignant cells.

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Introduction

Transmissible venereal tumor (TVT) also knowns as infectious sarcoma, venereal granuloma, transmissible lymphosarcoma or Sticker tumor, is the most common genital tumor in dogs (Özyiğit et. al., 2014; Paramjeet et. al., 2019). This tumor is mostly found in sexually active, free-circulating, young dogs in many regions of the world (Akkoc et. al., 2017). TVT, which has a higher incidence in tropical and subtropical regions, mostly affects dogs. In addition, it is known to affect other canids

*Corresponding Author: Emin Karakurt
E-mail: mehmeteminkarakurt@hotmail.com

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such as foxes, jackals and wolves (Stockmann et. al., 2011a). It has been reported that this tumor is seen with the same frequency in both female and male dogs (Özalp et. al., 2012). The tumor is transmitted from one dog to another by mating (Gupta and Sood, 2012). Although the tumor mostly affects the genital mucosa, it is also occurs in the oral, nasal and conjunctival mucosa (Guvenc et. al., 2002; Coskan et. al. 2011). Although the origin of TVT cells is unclear, it is classified as malignant round cell neoplasia with plasmacytoid or lymphocytic appearance (Flórez et. al., 2017). TVT has its own unique features. The origin of TVT is not shaped by the neoplastic transformation of normal cells. On the contrary, the development and evolutionary process of cells occur as a result of the transmission of clonal cells that pass from infected dogs to healthy dogs (Lima et. al., 2016). TVT cells need cellular disintegration due to their characteristic features before they settle on the mucous membrane. Once the tumor has settled, it can proliferate and spread to other locations in the body and eventually become metastatic. (Alzate et. al., 2019). TVT is highly invasive locally and the rate of metastasis is very low (Oguş and Özmen, 2018). TVT can metastasize to many tissues and organs such as liver, kidney, spleen, eyes, brain, pituitary gland, skin, subcutis, mesenteric lymph nodes and peritoneum (Tiwari et.al., 2016). Differential diagnosis of TVT is made based on cytological and histopathological findings (Oguş and Özmen, 2018). The tumor is evaluated in three phases histopathologically considering its developmental characteristics. These are briefly; proliferative (P), stable (S) and regression (R) phases (Stockmann et. al., 2011b; Setthawongsin et. al., 2019).

In this study, we aimed to evaluate the proliferative, metastatic and apoptotic capacities of TVT cases, which are in various phases of development, by using immunohistochemical markers.

Materials and Methods

Animals: The material of this study consisted of twelve female and six male dogs diagnosed with TVT brought to our department between 2007 and 2020 years. We divided these 18 animals into 3 phases (progression, stable and regression) including 6 of them.

Cytological Investigations: Diff-quick staining was applied to the smear taken from tumoral masses for cytological examinations.

Histopathological Investigations: Tumoral tissues from dogs were fixed in a 10% neutral buffered formaldehyde solution. After routine tissue procedures follow-up (dehydrated through graded %70-100 alcohols, xylol and embedded in paraffin) 5 µm thick sections were taken from paraffin blocks. For the examination of histopathological changes in tumoral tissues, Hematoxylin & Eosin (H&E) stain was applied to the sections. Tissue sections were investigated under a light microscope (Olympus Bx53) and photographed with Cell ^P Program (Olympus Soft Imaging Solutions GmbH, 3,4).

Immunohistochemical Investigations:

Immunohistochemical staining was applied on the tissues using the avidin-biotin immune peroxidase complex method. For immunohistochemical staining, sections of 4 µm thick from paraffin blocks were rehydrated. In order to prevent endogenous peroxidase activity, the sections were treated with 3% hydrogen peroxide solution for 15 minutes. Then, the microwave method (Citrate Buffer Solution pH 6 for 25 min; boiling for 15 min and cooling for 10 min at 800 watt) was applied to the sections to reveal the antigenic receptors. In order to prevent nonspecific staining, the sections were incubated for 30 min with Serum Blocking Solution (Genemed Biotechnologies REF 54-0003). Following treatment with Phosphate Buffered Salt Solution (PBS) with different antibodies; (PCNA: Santa Cruz, sc-56, Dilution Ratio: 1/100), (MMP-9: Santa Cruz, sc-393859, Dilution Ratio: 1/100), (p53: Novus Bio, SPM590, Dilution Ratio: 1/200), (Bax: Bioss, bs-0127R, Dilution Ratio: 1/200) and (Bcl-2: Bioss, bs-0032R, Dilution Ratio: 1/200) were incubated for overnight. The sections were washed 3 times in PBS solution for 5 minutes, and the biotinylated temperature for 30 minutes. After washing in PBS (3-5 min), all sections were incubated with

Table 1. Statistical comparison of PCNA, MMP-9, p53, Bax and Bcl-2 immune-positive reactions between progression, regression and stable TVT groups

Groups	PCNA	MMP-9	p53	Bax	Bcl-2
Progression	2.83 ± 0.40[a]	2.66 ± 0.51[a]	2.50 ± 0.54[a]	1.33 ± 0.51[a]	2.83 ± 0.40[a]
Regression	1.33 ± 0.51[b]	1.16 ± 0.40[b]	1.33 ± 0.51[b]	2.66 ± 0.51[b]	1.33 ± 0.51[b]
Stable	2.33 ± 0.51[c]	2.00 ± 0.63[c]	1.83 ± 0.40[c]	2.33 ± 0.51[b]	2.16 ± 0.40[c]
	P <0.05	P<0.05	P< 0.05	Pp <0.05	P <0.05

a, b, c express the differences between the groups (p <0.05).

(peroxidase-bound Streptavidin-Peroxidase (HRP) Genemed Biotechnologies REF 54-0003) for 30 minutes. A solution of 3,3'-Diaminobenzidine (DAB) (Genemed Biotechnologies REF 10-0048) were used as colour revealing substrate. The sections were stained with Mayer Hematoxylin and coated with immune mount. The sections prepared after the covering were examined under a light microscope (Olympos Bx53) and photographed via the Cell^P program (Olympos Soft Imaging Solutions Gmbh, 3,4).

Statistical analysis: PCNA, MMP-9, p53, Bax and Bcl-2 immune-positive reactions were evaluated under a light microscope negative (-), mild (+), moderate (++) and severe (+++). The data obtained were analyzed with SPSS 20.00 program. The difference between the groups was determined by Kruskal Wallis, one of the nonparametric tests, and the group that created the difference was determined by the Mann Whitney U test (p <0.05).

Results

Macroscopic results: We determined that tumoral masses have cauliflower appearance. In addition, we detected quite common hemorrhage and ulcerative areas. The tumor was easily crumbly and had a very fragile structure (Figure 1).

Cytological results: In the Diff-quick staining performed on smears taken from tumoral masses, we observed the presence of plasmacytoid tumoral cells with large and vacuolar cytoplasm. It was noteworthy that tumoral cells had a distinct nucleus. Nuclei were eccentrically located. In addition to tumoral cells, the presence of a relatively small number of neutrophil leukocytes was also recorded by us (Figure 2).

Figure 1. Macroscopic view of tumoral mass, which is quite hemorrhagic.

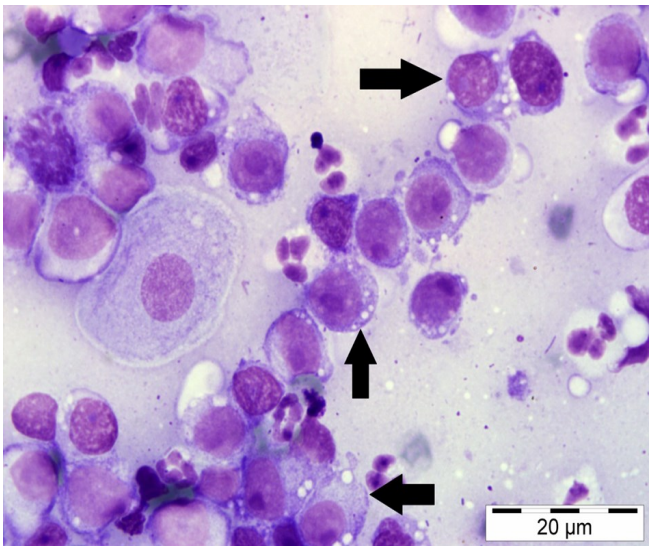

Figure 2. Plasmacytoid tumoral cells with large and vacuolar cytoplasm with an eccentrically located nucleus (arrows), Diff-quick stain, Bar= 20µm.

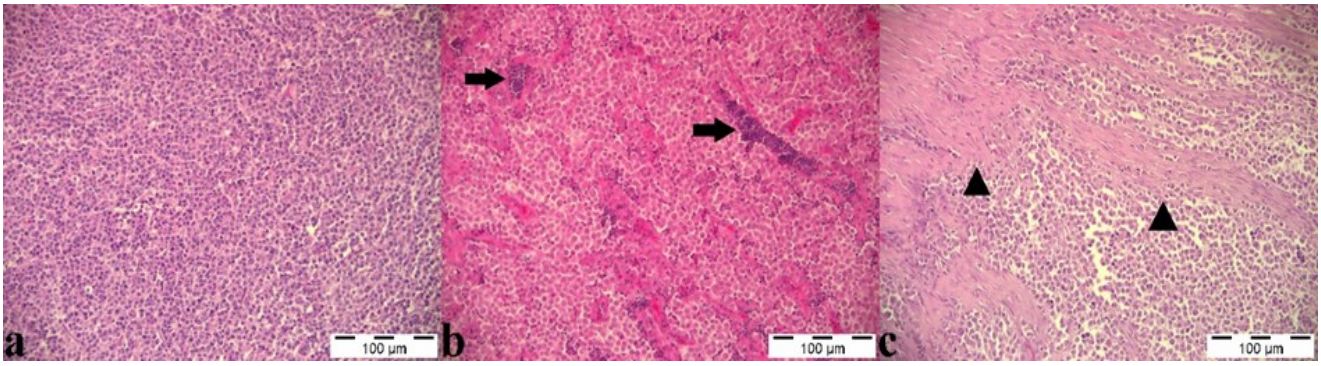

Figure 3. (a) Progression phase TVT case, increase in cellularity and mitotic figures, H&E, Bar= 100µm. **(b)** Stable phase TVT case, lymphocytes infiltrating the tumoral tissue (arrows), H&E, Bar= 100µm. **(c)** Regression phase TVT case, decreased cellularity, increased connective tissue (arrowheads), H&E, Bar= 100µm.

secondary antibody (Genemed Biotechnologies REF 54-0003) was applied to them at room

Hematoxylin & eosin results: In the histopathological examination of tumoral masses; In TVT cases in the progression phase, we found that the tumor cells surrounded by thin fibrous capsules increased significantly and the mitotic figures were abundant (six animals). In addition, we observed areas of intense necrosis and bleeding (Figure 3a). In the stable phase TVT cases, the presence of lymphocytic cells infiltrated into the tumoral foci surrounded by a fibrous capsule attracted our attention (six animals) (Figure 3b). In the regression phase TVT cases, we found that cellularity decreased significantly and fibrous connective tissue increased significantly (six animals) (Figure 3c).

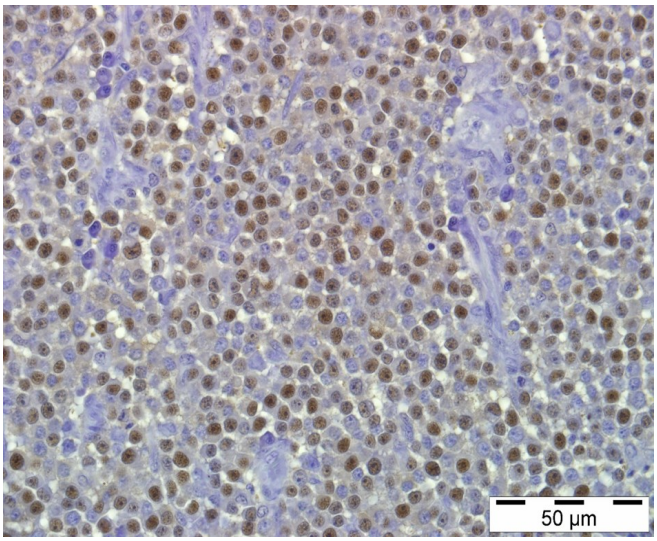

Figure 4. Progression phase TVT case, PCNA immunoreactivity in the nucleus of tumoral cells, IHC, Bar= 50µm.

Immunohistochemical results: Statistical comparison of PCNA, MMP-9, p53, Bax and Bcl-2 immune-positive reactions between groups as shown in Table 1. We observed PCNA immunopositive reactions mostly in TVT cases in the progression phase. PCNA positive reaction was especially in the nucleus of the tumoral cells and brown color (Figure 4). MMP-9 immunopositive reaction was also stronger in TVT cases at the phase of progression, similar to PCNA.

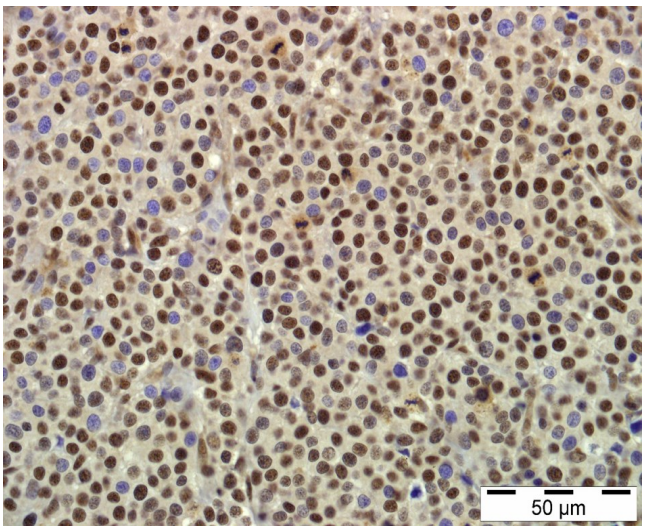

Figure 5. Progression phase TVT case, MMP-9 immune positive reaction in the cytoplasm and nucleus of tumoral cells, IHC, Bar= 50µm.

MMP-9 immunoreactivity was seen predominantly in the nucleus of tumoral cells, but less frequently in the cytoplasm (Figure 5). P53 immunoreactivity showed a more pronounced increase, especially in cases of progression TVT. While p53 immunopositive reactions were observed especially in the cytoplasm of tumoral cells, spontaneous positive

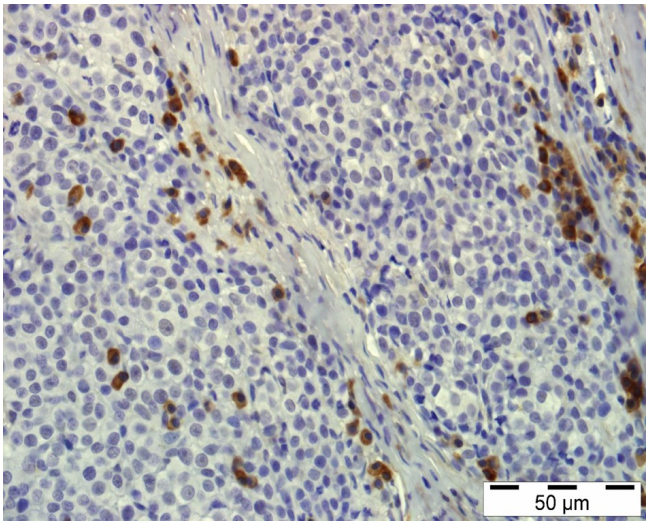

Figure 6. Progression phase TVT case, p53 expression in the cytoplasm and nucleus of tumoral cells, IHC, Bar= 50µm.

reactions were observed in the nucleus (Figure 6). The cytoplasmic Bax immunopositive reaction was also stronger in TVT cases at the phase of regression (Figure 7). We observed Bcl-2 immunoreactivity especially in the cytoplasm and membrane of tumoral cells. The expression of Bcl-2 was higher as to Bax expression in progression cases than regression cases (Figure 8).

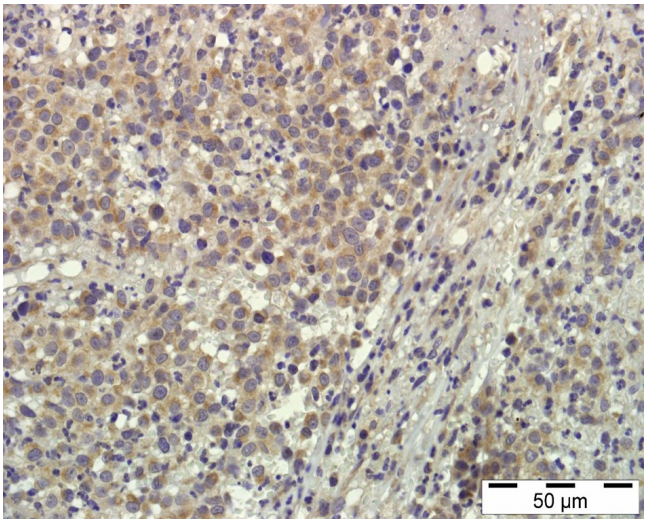

Figure 7. Regression phase TVT case, Bax immunoreactivity in the cytoplasm of tumoral cells, IHC, Bar= 50µm.

Discussion and Conclusion

Detection of tumor cell division rate is an important parameter in determining tumor aggressiveness and prognosis (Tiwari et al., 2016). Proliferating cell nuclear antigen (PCNA), which is used to calculate the proliferation index in animal neoplasms, is associated with DNA replication and repair (Gupta and Sood, 2012). Although it is present at all of the cell cycle stages, it reaches the maximum level in the S phase (Madewell, 2001). The increase in PCNA expression is valuable in terms of high proliferation rate and degree of aggressiveness (Paramjeet et al., 2019). In our study, we evaluated a highly reliable marker PCNA expression in order to compare the stages of development of TVT cases in terms of cell proliferation (Chu et al., 2001). Similar to previous studies (Chu et al., 2001; Tiwari et al., 2016) we detected PCNA expression at a

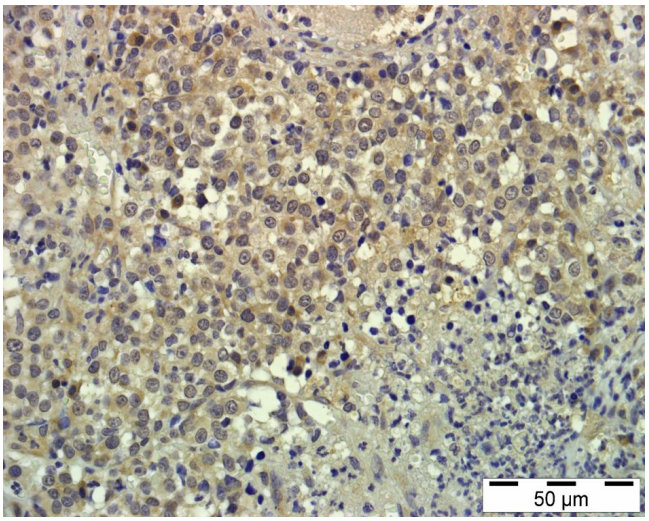

Figure 8. Progression phase TVT case, Bcl-2 immunoreactivity in the cytoplasm and membrane of tumoral cells, IHC, Bar= 50µm.

statistically higher rate in TVT cases at the progression stage compared to regression and stable stage cases. The PCNA reaction was in the nucleus of the tumoral cells and moderate to severe in accordance with the literature data (Gupta and Sood, 2012; Lokesh et al., 2014; Tiwari et al., 2016; Paramjeet et al., 2019). According to the results of our study, we thought that PCNA is a very reliable marker in determining tumor aggression and prognosis in TVT cases.

The most vital task of the extracellular matrix (ECM) is to protect the tissues with their specific and mechanical properties (Oguş and Özmen, 2018). Matrix metalloproteinases (MMPs) play a role in the degradation of ECM proteins.

Elimination of the ECM proteins that surround the tumor cells is an important step for metastasis. (Akkoc et. al., 2017). Increased concentrations of MMPs have been associated with invasion, metastasis and poor prognosis in many malignancies of humans (Loukopoulos et al., 2003; Aresu et al., 2011). MMP-2 and MMP-9 are the most studied proteases in cancer biology (Akkoc et al., 2017). Although TVT has a very low capacity to metastasize, it can metastasize to many tissues and organs such as liver, kidney, spleen, eyes, brain, pituitary gland, skin, subcutis, mesenteric lymph nodes and peritoneum (Tiwari et al., 2016). In our study, we evaluated MMP -9 expressions immunohistochemically to compare the metastatic capacities of different stages of the tumor. In accordance with previous studies, all TVT cases responded positively with MMP-9 (Akkoc et al. 2017). Unlike this study, MMP-9 expression was observed especially in the nucleus of tumoral cells, while cytoplasmic reactions were milder than nuclear reactions (Akkoc et al. 2017). In addition, we detected MMP-9 immunoreactivity especially in cases of progressive stage. Unfortunately, we do not have sufficient information on whether these cases have metastasized because of insufficient information from the animal owners after the surgical operations. However, in cases where MMP-9 expressions are high, we believe that especially immune system suppressed animals can metastasize to distant tissues. (Özalp et al., 2012).

When any DNA damage occurs in normal cells, an increase in the p53 gene is formed, and the cell cycle is blocked at the G1 stage and the damaged cell is given time for repair. If the damage is quite severe, apoptosis, a programmed cell death rather than repair, is activated (Moro et al., 2010). p53, also known as the guardian of the genome, plays an important role in regulating the cell cycle and maintaining the integrity of the genome (Santos et al., 2010). Mutations in the p53 gene are quite common in different types of tumors, and as a result of these mutations, the gene cannot perform its normal functions. Thus, the DNA damage in the cell cannot be repaired or if the damage is very severe, the apoptosis mechanism cannot be activated. (Gerardi et al., 2014). The p53 protein is quickly eliminated as it has a short half-life of 20 minutes. The half-life of the mutant p53 protein is several hours, which allows it to be detected by immunohistochemical methods (Tiwari et al., 2014). In our study, we preferred the mutant p53 gene compared to the normal p53 gene due to its long half -life. In accordance with previous literature data, we detected p53 immunoreactivity in the cytoplasm and nucleus of tumoral cells (Coskan et al., 2011; Gupta and Sood, 2012; Gerardi et al., 2014). Stockmann et al.2011b reported that they encountered a p53 positive reaction at all stages, regardless of the stage of development of the tumor. Moro et al. 2010 detected more p53 immunopositive cells in the regression phase in TVT. In our study, we determined more mutant p53 gene expressions especially in progressive TVT cases similar to previous studies (Santos et al., 2008; Gerardi et al., 2014). In the light of these results, we thought that mutations in the p53 gene block apoptosis and trigger uncontrolled cell proliferation (Santos et al. 2008; Moro et al., 2010). It supports our opinion that especially the cases in the progression stage show more severe mutant p53 gene expression. We have interpreted that mutations in the p53 gene in TVT of dogs can affect the development and aggressiveness of this tumor.

The intrinsic pathway is regulated by the B cell lymphoma 2 (Bcl-2) family. The protein X associated with BCL2 (Bax) is a proapoptotic protein that triggers mitochondrial membrane permeability in response to apoptotic stimuli. In contrast, Bcl-2 is an anti-apoptotic protein that protects cell death (Alzate et al., 2019). In the antiapoptotic effect of Bcl-2, it is important to stabilize the mitochondrial membrane and prevent the release of cytochrome-c (Lokesh et al.; 2014). Therefore, the balance between these two proteins is very important (Setthawongsin et al., 2019). Over-expression of Bcl-2 is seriously associated with the survival of malignant tumor cells, especially their aggressiveness and poor prognosis (Özyiğit et al., 2014). Also, overexpression of Bcl-2 may be related to the mutant p53 gene (Stockmann et al. 2011a). In our study, similar to this data, mutant p53 and Bcl-2 expressions were highest in progressive TVT cases. Stockmann et al. 2011b reported that Bcl-2 overexpression is independent of TVT development phases. Lokesh et al. 2014 reported that they could not detect the Bcl-2 reaction in TVT cases. Similar to previous studies (Özyiğit et al., 2014; Setthawongsin et al., 2019), we observed that Bcl-2 expression was more severe in progressive TVT cases and Bax expression was more severe in regressive TVT cases.

In conclusion, in our study, we evaluated the proliferation, apoptosis and metastasis properties of TVT by various markers such as PCNA, MMP-9, p53, Bax and Bcl-2. We evaluated these markers in detail in cases where we divided them into three as progression, stable and regression phases according to their histopathological features and compared the cases statistically with each other. As a result of the evaluations, we thought that the mentioned markers are very useful for understanding the prognosis of TVT, the tumor behavior and the survival of the cells. From this aspect, we believed that the data obtained from this study will contribute to the literature.

References

Akkoc, A., Nak, D., Demirer, A., & Şimşek, G. (2017). Immunocharacterization of matrix metalloproteinase-2 and matrix metalloproteinase-9 in canine transmissible venereal tumors. *Biotechnic & Histochemistry, 92*(2), 100-106.

Alzate, J. M., Montoya-Florez, L. M., Pérez, J.E., Rocha, N. S., & Pedraza-Ordonez, F. J. (2019). The role of the multi-drug resistance 1, p53, b cell lymphoma 2, and bcl 2-associated X genes in the biologic behavior and chemotherapeutic resistance of canine transmissible venereal tumors. *Veterinary Clinical Pathology, 48*(4), 730 -739.

Aresu, L., Giantin, M., Morello, E., Vascellari, M., Castagnaro, M., Lopparelli, R., Zancanella, V., Granato, A., Garbisa, S., Aricò, A., Bradaschia, A., Mutinelli, F., & Dacasto, M. (2011). Matrix metalloproteinases and their inhibitors in canine mammary tumors. *BMC Veterinary Research,* 7, 33.

Chu, R. M., Lin, C. Y., Liu, C. C., Yang, S. Y., Hsiao, Y. W., Hung, S. W., Pao, H. N., & Liao, K. W. (2001). Proliferation characteristics of canine transmissible venereal tumor. *Anticancer Research. 21*(6A), 4017-4024.

Coskan, A. S., Alcigir, M. E., & Atalay Vural, S. (2011). Pathomorphological and immunohistochemical findings in a case of extragenital canine transmissible venereal tumour. *Bulgarian Journal of Veterinary Medicine, 14*(4), 252-256.

Flórez, M. M., Fêo, H. B., da Silva, G. N., Yamatogi, R. S., Aguiar, A. J., Araújo Jr, J. P., & Rocha, N. S. (2017). Cell cycle kinetics, apoptosis rates and gene expressions of MDR-1, TP53, BCL-2 and BAX in transmissible venereal tumour cells and their association with therapy response. *Veterinary and Comparative Oncology, 15*(3), 793-807.

Gerardi, D. G., Tinucci-Costa, M. Silveira, A. C. T., & Moro, J. V. (2014). Expression of P-glycoprotein, multidrug resistance-associated protein, glutathione-S-transferase pi and p53 in canine transmissible venereal tumor. *Pesquisa Veterinária Brasileira, 34*(1), 71-78.

Gupta, K. & Sood, N. K. (2012). Pathological and immunohistochemical studies on rare cases of primary extra-genital transmissible venereal tumours in the mammary gland. *Veterinarni Medicina, 57*(4), 198-206.

Guvenc, T., Haligur, M., Orman, M. N., & Haziroglu, R. (2002). Mitosis and apoptosis in canine cutaneous histiocytoma and transmissible venereal tumour. *Acta Veterinaria Hungarica, 50*(3), 315-321.

Lima, C. R. O., Faleiro, M. B. R., Rabelo, R. E., Vulcani, V. A. S., Rubini, M. R., Torres, F. A. G., & Moura, V. M. B. D. (2016). Insertion of the LINE-1 element in the C-MYC gene and immunoreactivity of C-MYC, p53, p21 and p27 proteins in different morphological patterns of the canine TVT. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 68*(3), 658-666.

Lokesh, J. V., Kurade, N. P., Shivakumar, M. U., Sharma, A. K., & Maiti, S. K. (2014). Evaluation of BcL-2 and PCNA expression and mitotic index in spontaneous canine tumors. *Advances in Animal and Veterinary Sciences, 2*(1), 63-66.

Loukopoulos, P., Mungall, B. A., Straw, R. C., Thornton, J. R., & Robinson, W. F. (2003). Matrix metalloproteinase-2 and -9 involvement in canine tumors. *Veterinary Pathology, 40*(4), 382-

Madewell, B. R. (2001). Cellular proliferation in tumors: a review of methods, interpretation, and clinical applications. *Journal of Veterinary Internal Medicine, 15*(4), 334-340.

Moro, J. V., Tinucci-Costa, M., Silveira, A. C. T., Gerardi, D. G., & Alessi, A. C. (2010). Reactivity of p53 protein in canine transmissible venereal tumor. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 62*(2), 318-323.

Oguş, E. & Özmen, Ö. (2018). Immunohistochemical determination of the matrix metalloproteinase-2 and -7 expression in transmissible venereal tumor in dogs. *Mehmet Akif Ersoy Üniversitesi Veteriner Fakültesi Dergisi, 3*(2), 106-110.

Özalp, G. R., Zik, B., Bastan, A., Peker, S., Özdemir-Salci, E. S., Bastan, I., Darbaz, I., Salar, S., & Karakas, K. (2012). Vincristine modulates the expression of Ki67 and apoptosis in naturally occurring canine transmissible venereal tumor (TVT). *Biotechnic & Histochemistry, 87*(5), 325-330.

Özyiğit, M. Ö., Nak, D, Akşit, H., İnan Öztürkoğlu, S. Şimşek, G. Uzabacı, E., Nak. Y, & Seyrek, K. (2014). The effects of vincristine sulfate on expression of galectin-3, Bcl-2, and carbohydrate structures specific for EEL, GSL-1, and RCA-1 lectins in bitches with naturally occuring canine transmissible venereal tumor. *Turkish Journal of Veterinary and Animal Sciences,* 38, 331-338.

Paramjeet, Jangir, B. L., Lather, D., Saharan, S., & Jakhar, K. K. (2019). Pathomorphological and immunohistochemical studies on transmissible venereal tumours in dogs. *Haryana Veterinarian, 58*(1), 82-85.

Santos, F. G. A., Vasconcelos, A. C., Nunes, J. E. S., Cassali, G. D., Paixão, T. A., Martins, A. S., Silva, S. S., Martins, R. F., & Moro, L. (2008). Apoptosis in the transplanted canine transmissible venereal tumor during growth and regression phases. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia,60*(3), 607-612.

Setthawongsin, C., Teewasutrakul, P., Tangkawattana, S., Techangamsuwan, S., & Rungsipipat, A. (2019). Conventional-vincristine sulfate vs. modified protocol of vincristine sulfate and l-asparaginase in canine transmissible venereal tumor. *Frontiers in Veterinary Science,* 6, 300.

Stockmann, D., Ferrari, H. F., Andrade, A. L., Rodrigo, A. L., Cardoso, T. C., & Luvizotto, M. C. (2011a). Canine Transmissible Venereal Tumors: Aspects Related to Programmed Cell Death. *Brazilian Journal of Veterinary Pathology, 4*(1), 67-75.

Stockmann, D., Ferrari, H. F., Andrade, A. L., Cardoso, T. C., & Luvizotto, M. C. (2011b). Detection of the tumour suppressor gene TP53 and expression of p53, Bcl-2 and p63 proteins in canine transmissible venereal tumour. *Veterinary and Comparative Oncology, 9*(4), 251 -259.

Tiwari, K. P., Bhaiyat, M. I., Chikweto, A., Inga, A., Sharma, R. N., & Pawaiya, R. V. S. (2014). Immunohistochemical detection of p53 tumor suppressor protein in round cell tumors of dogs in Grenada, West Indies. *Advances in Animal and Veterinary Sciences, 2*(3S), 23-27.

Tiwari, K. P., Bhaiyat, M. I., Chikweto, A., Sharma, R. N., Younger, C. H., & Pawaiya, R. V. S. (2016). Expression pattern of proliferating cell nuclear antigen (PCNA) in round cell tumors of dogs from Grenada, West Indies. *Indian Journal of Veterinary Pathology, 40*(2), 144-147.

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Epidemiology of coccidiosis and effects of the infection on some clinical and hematological examination findings in calves

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Bülent Elitok

Afyon Kocatepe University, Veterinary Faculty, Department of Internal Medicine, 03200, Afyonkarahisar, Turkey. Elitok B.: ORCID: 0000-0003-3336-4479.

ABSTRACT

The aim of this study was to determine the effects of *Eimeria* species on hematological parameters in calves with coccidiosis in Afyonkarahisar/Turkey province. This study was carried out in a group consisting of 28 calves (12 females and 16 males) with coccidiosis confirmed by microscopic examinations in faeces. Some clinical and hematological measurements were performed on all the calves. Results of the present study shown that, there was statistically significant decrease ($p < 0.05$) in average of erythrocyte count, hemoglobin concentration, hematocrit value, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, lymphocyte and eosinophil count compared with healthy animals. While, there was statistically significant increase ($p < 0.05$) in the mean of total leukocyte and neutrophil count. Since coccidiosis is a frequently encountered disease, clinical and hematological findings obtained from this study will provide beneficial information to the practice and scientific community as a reference.

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Introduction

Eimeria ssp. is one of the biggest problems affecting calves, especially 4-7 weeks (Bangoura and Daugschies, 2007; Philippe et al., 2014; Tavassoli et al, 2018). This disease causes economic losses due to high mortality and morbidity, low growth and treatment costs (Kaya, 2004; Arslan et al., 2012). The disease exhibits an increasing incidence in conditions such as breeding type, barn type, hygiene status, nutrition, age of the animal, enteric infections, transplants, weaning, birth season and periparturient period (Jolley and Bardsley, 2006; Arslan et al., 2012; Tavassoli et al, 2018).

Eimeria species are transmitted by fecal-oral route through feces containing oocytes and are more common in young animals (Lassen et al., 2009; Sudhakara et al., 2015; Kim et al., 2018). Clinically, it is accompanied by watery and bloody diarrhea, fever, dehydration, malnutrition, anorexia and weight loss, and it has a subclinical

*Corresponding Author: Bülent Elitok
E-mail: elitok1969@hotmail.com

https://dergipark.org.tr/tr/pub/http-www-jivs-net

course in adult cattle (Cornelissen et al., 1995; Koutny et al., 2012; Kim et al., 2018).

Significant changes are also observed in hematological parameters in coccidiosis-infected calves (Begum, 1981; Anwar et al., 1999). Baker et al. (1998) reported that there were significant differences between the races in terms of *Eimeria* infection and hematocrit levels (HTC), and that infection caused a decrease in HTC value. However, in another study (Liang et al., 2001), there was a contradiction between the HTC level and the finding of anemia, and it was concluded that this was due to the mild hemorrhage. In another study (Al-Dahwi et al., 2006), a reduction in the number of peripheral eosinophil was reported in the presence of *Eimeria* infection. In another study (Rakhshandehroom et al., 2013), neutrophil and HTC levels were increased but eosinophil counts did not change in coccidiosis cases.

As can be seen from the above-mentioned studies, the fact that the findings of the present studies differ in terms of hematological parameters leads to the conclusion that regional research in coccidiosis cases may give more accurate results. The aim of this study was to determine the effects of *Eimeria* species on hematological parameters in coccidiosis calves in Afyonkarahisar province.

Materials and methods

This study was carried out in 28 calves (12 females and 16 males) with coccidiosis, which were microscopically determined to have *Eimeria* spp. oocysts in their stools, as a result of screening from 98 animals between 4-8 weeks of age in different areas in Afyonkarahisar province. While 28 calves with coccidiosis constituted the study group (SG), 28 calves found to be clinically healthy at the same age constituted the control group (CG).

Clinical and hematological examinations: Routine clinical examinations (body temperature, heart beat and respiratory frequency) were performed in the calves according to the previously reported method (Blood and Radostits, 2007), and blood samples taken from the Vena jugularis of the animals were measured. Hematologically, erythrocyte (RBC), total leukocyte (WBC), hematocrit (HCT), hemoglobin (HB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MHC), mean corpuscular hemoglobin concentration (MCHC), lymphocyte (LENF), neutrophil (NOTR), eosinophil (EOS), monocyte (MON) and basophil (BAS) were measured using Chemray Brand blood count device using commercial test kits.

Stool Examinations: Fresh samples were taken from the feces of the animals and put into plastic containers and the samples were taken and the feces were examined. In the samples taken in the stool examinations, firstly native examination and then using the flotation method (Blood and Radostits, 2007) under the light microscope in terms of *Eimeria* spp.

Statistical analysis: Differences between the hematological parameters of the study and control animals were calculated by using Student-t test in SPSS for Windows Version 18.1 package program and $p < 0.05$ was considered statistically significant.

Results

Although no abnormal color change was observed in the visible mucosa, 12 (42.8%) of the animals in the study group had mild anemic appearance. Microscopic examinations of fecal samples showed significant erythrocyte in all study group animals, whereas only three animals (10.7%) in the control group showed a small number of erythrocytes. In terms of color, it was found that the feces of the study group were darker, there were traces of blood in 5 of them (17.8%), moderate diarrhea was formed in these animals, whereas the feces of the control group animals were lighter and none of them showed bleeding signs and did not develop diarrhea. Although clinical examination revealed that lymph nodes were normal in both groups, it was observed that the study group animals were weaker compared to the control group. The body temperature (T), heart (P) and respiratory (R) frequencies of the groups are shown in Table 1. When Table 1 is examined; although there was no

Table 1. Clinical examinations of control (CG) and study group (SG) animals

Groups	BT (°C)	HR (Beat/min)	RR (frequency/min)
	X ± SEM	X ± SEM	X ± SEM
Control	39.20 ± 0.20	76.00 ± 3.00[b]	58.00 ± 3.00[b]
Study	39.10 ± 0.30	88.00 ± 4.00[a]	66.00 ± 6.00[a]

[a, b.] The difference between the means indicated by different letters in the same column is statistically significant (p<0.05). BT = Body temperature, HR = Heart rate, RR = Respiratory rate

significant difference between the groups in terms of body temperature (p> 0.05), P beat and R frequency averages were found to be significantly higher in the study group animals (p <0.05).

The hematological examination findings of the control and study groups are shown in Table 2. and Table 3. When Table 2 and Table 3 are examined; average of erythrocyte, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, lymphocyte and eosinophil was statistically significantly (p <0.05) lower than those of healthy animals, while the mean of total leukocyte and neutrophil was statistically significantly higher (p <0.05) in SG animals. There was no difference between the groups in terms of basophil (p>0.05).

Discussion and conclusion

The proportion of coccidiosis we screened in this study was 28.57% according to microscopic detection of *Eimeria* spp. oocysts in fecal samples. This rate we obtained in this study was in agreement with some investigators (Cicek et al., 2007) reported that the prevalence of coccidiosis was 27.23%.

In our study, the increase in respiratory and heart rates in calves with coccidiosis may be directly related to the decrease in HB and RBC levels. As a matter of fact, the importance of HB in the transport of CO2 is great and in the shortage of HB formed in anemia cases, pulmonary ventilation of CO2 increases and its frequency increases (Blumgart and Altschule, 1948). In addition, the increase in heart rate is a result of efforts to compensate for O2 deficiency in the blood (Liang et al., 2001).

Eimeria agents proliferate merogonic in intestinal epithelial cells. Pathologically, destruction of intestinal epithelial cells, villus loss, disruption of absorption, enteritis, dehydration and diarrhea occurs (Levine, 1985). Depending on the magnitude of the damage caused by the intestine and the population of the causative agents, it can lead to mild to severe bleeding and ultimately anemia (Parker and Jones, 1990; Blood and Radostits, 2007). When we compared the control group with RBC, HB, HTC, MCV, MCH and MCHC averages, it was caused by the mentioned damage. Similar findings Arslan et al. (2012), but there are many studies reporting the same or different findings. For example, Anwar et al. (1999) observed a significant decrease in RBC in cases of coccidiosis and attributed this to blood loss from hemorrhagic intestinal mucosa and bloody diarrhea. In our present study, the decrease in RBC also decreased proportionally with the

Table 2. Hematological examination findings of control (CG) and study group (SG) animals

Groups	WBC (m/mm3)	RBC (m/mm3)	HB (g/dl)	HTC (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
	X ± SEM	X ± SEM	X ± SEM	X ± SEM	X ± SEM	X ± SEM	X ± SEM
CG	8.24±2.16[b]	7.42±2.08[a]	10.40±2.10[a]	36.24±4.00[a]	48.36±4.00[a]	14.03±2.00[a]	28.68±2.30[a]
SG	9.37±3.20[a]	5.40±1.30[b]	7.28±1.20[b]	32.40±3.30[b]	44.43±3.10[b]	13.28±2.20[b]	22.54±2.10[b]

Table 3. Differential leukocyte count of control (CG) and study group (SG) animals

Groups	Lymphocyte (%)	Neutrophil (%)	Eosinophil (%)	Monocyte (%)
	X ± SEM	X ± SEM	X ± SEM	X ± SEM
CG	52.10 ± 4.00[a]	36.20 ± 3.20[b]	7.00 ± 0.30[a]	3.10 ± 0.10
SG	38.30 ± 5.00[b]	56.40 ± 6.00[a]	4.40 ± 0.20[b]	3.00 ± 0.20

percentage of HB HTC values were directly related to erythrocyte number. Similar observations have been identified in studies conducted by another investigator (Begum, 1981). However, contrary to the decrease in HB and HTC values, there are also studies that give opinions. Pout (1965) argued that these two parameters did not change in sheep coccidiosis. In our study obtained low levels of MCV, MCH and MCHC, hemopoietic factors refer to macrocytic anemia developed as a result of insufficiency of erythrocyte production reported by Shommein and Osman (1980) who claimed that hypochromic anemia would develop during the course of coccidiosis.

In our current study, the decrease in EOS and LENF averages in the study group and the increase in NOTR and WBC averages were consistent with the findings (Rama et al., 1978; Malik, 1987). It has been reported that the decrease in LENF levels may also be caused by globulins under the control of ACTH hormones on lymphoid tissues and lymphocytes (Begum, 1981).

In our study, no difference was found between the two groups in terms of MON levels. Some investigators (Anwar et al., 1999) have reported that monocytes are usually increased in chronic infections, while coccidiosis is an acute infection.

Since coccidiosis is a common disease and hematological parameters show significant changes, and since there is no previous studies in this area, especially in our region, we think that our current study will make significant contributions to the scientific community due to both the practice and the reference.

References

Al-Dahwi, Z., Mayberry, L. F., Conder, G. A., & Bristol, J. R. (2006). Suppression of extraintestinal and intestinal Nippostrongylus brasiliensis- induced eosinophilia by *Eimeria* nieschulzi. *Journal of Parasitology, 9* (5), 962-970.

Anwar, A. H., Kazmi, S. I. H., & Khan, M. N. (1999). Deneysel olarak oluşan koksidiyozun manda buzağılarının bazı kan parametreleri üzerine etkisi. *Pakistan Journal of Biological Science, 2*(3), 1024-1026.

Arslan, M. Ö., Sarı, B., Kara, M., Taşçı, G. T., & Ekinci, A., & Gündüz, N. (2012). Research on the Prevalence of *Eimeria* and Cryptosporidium Species in Cows in Periparturient Period in Kars Region. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi,* 18, 65-70.

Baker, R. L, Mwamachi, D. M. J., Audho, J. O., Aduda, E. O., & Thorpe, W. (1998). Resistance of galla and small east African goats in the sub-humid tropics to gastrointestinal nematode infections and the periparturient rise in faecal egg counts. *Veterinary Parasitology*, 79, 53-64.

Bangoura, B., & Daugschies, A. (2007). Parasitological and clinical parameters of experimental *Eimeria zuernii* infection in calves and influence on weight gain and haemogram. *Parasitology Research,* 100, 1331–1340.

Begum, N. (1981). Study on hematology and

References

electrolyte balance in sheep infected with pathogenic coccidial oocysts. *M.Sc. Thesis*, University of Agriculture, Faisalabad, Pakistan.

Blood, D. C., & Radostits, O. M. (2007). *Veterinary Medicine*. London. UK: Bailliere Tindall,

Blumgart, H. L., & Altschule, M. D. (1948).Clinical significance of cardiac and respiratory adjustments in crronic anemia. *Blood, 3*(4), 329-348.

Cicek, H., Sevimli, F., Kozan, E., Köse, M., Eser, M., & Doğan N. (2007). Prevalence of coccidia in beef cattle in western Turkey. *Parasitology Research,* 101: 1239-1243.

Cornelissen, A. W., Verstegen, R., van den Brand, H., Perie, N. M., Eysker, M., Lam, T. J., & Pijpers, A. (1995). An observational study of Eimeria species in housed cattle on dutch dairy farms.*Veterinary Parasitology,* 56, 7-16.

Jolley, W. R., & Bardsley, K. D. (2006). Ruminant coccidiosis. Veterinary Clinics of North America: food animal Practice, 22,613-621.

Kaya, G. (2004). Prevalence of Eimeria species in lambs in Antakya province. *Turkish Journal of Veterinary and Animal Science,* 28, 687-692.

Kim, H. C., Choe, C., Kim, S., Chae, J. S., Yu, D. H., Park, J., & Choi, K. S. (2018). Epidemiological survey on Eimeria spp. associated with diarrhea in pre-weaned native Korean calves. *The Korean journal of parasitology, 56*(6), 619-623.

Koutny H, Joachim A, Tichy A, & Baumgartner W. (2012). Bovine Eimeria species in Austria. *Parasitology Research,* 110, 1893-1901.

Lassen, B., Viltrop, A., Raaperi, K., & Järvis, T. (2009). Eimeria and Cryptosporidium in Estonian dairy farms in regard to age, species, and diarrhoea. *Veterinar Parasitology,* 166, 212-219.

Levine, N. D. (1985). *Veterinary Protozoology.* Ames, USA: Iowa State University Press.

Liang, Y., Hua, Z., Liang, X., Xu, Q., & Lu, G. (2001). The crystal structure of bar-headed goose hemoglobin in deoxy form: the allosteric mechanism of a hemoglobin species with high oxygen affinity. *Journal of Molecular Biology, 313*(1), 123-37.

Malik, A. A. (1987). Effects of experimentally induced coccidiosis on some blood parameters and productivity of sheep. *M.Sc. Thesis*, University of Agriculture, Faisalabad, Pakistan.

Parker, R. J., & Jones, G. W. (1990). Destruction of bovine coccidial oocysts in simulated cattle yards *Eimeria zuernii* by dry tropical winter weather. *Veterinary Parasitology,* 35, 269-272.

Philippe, P., Alzieu, J. P., Taylor, M. A., & Dorchies, P. (2014). Comparative efficacy of diclazuril (Vecoxan®) and toltrazuril (Baycox bovis®) against natural infections of Eimeria bovis and *Eimeria zuernii* in French calves. *Veterinry Parasitology,* 206, 129-137.

Pout, D. P. (1965). Coccidiosis in lambs. The *Veterinary Record*, 77, 887-888.

Rakhshandehroom, E, Saeed, N, Mostafa, R. S., Ghane, M., Alavi, A. M. (2013). Caprine coccidiosis: the effects of induced infection on certain blood parameters. *Veterinarski Arhiv, 83*(6),623-631.

Rama, S. P., Singh, C. D. N., Sinha, B. K., & Prasad, L. N. (1978). Experimental coccidiosis in sheep: Haematologiacl observations. *Indian Veterinary Medicine Journal,* 2, 192-199.

Shommein, A. M. & Osman, H. M. (1980). The effect of goat coccidiosis on certain blood components. *Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux,* 33, 371-375.

Sudhakara, R. B., Sivajothi, S., & Rayulu, V. C. (2015). Clinical coccidiosis in adult cattle. *Journal of Parasitic Diseases,* 39: 557-559.

Tavassoli, M., Dalir-Naghadeh, B., Valipour, S., & Maghsoudlo, M. (2018). Prevalence of gastrointestinal parasites in water buffalo (Bubalus bubalis) calves raised with cattle in smallholder farming system in the Northwest of Iran. *Acta Veterinary Eurasia*, 44, 6-11.

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Estimation of *schistosomiosis* infection rate in the sheep in Shiraz region, Fars province, Iran

Research Article

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Gholamreza Karimi[1*], Mohammad Abdigoudarzi[2]

1. Razi Vaccine and Serum Research Institute, Department of Parasitology, Agricultural Research, Education and Extension Organization, (AREEO), Tehran, Iran. **2.** Razi Vaccine and Serum Research Institute, Department of Parasitology, Reference Laboratory for ticks and tick borne research, Agricultural Research, Education and Extension Organization, (AREEO) Tehran, Iran.

Karimi Gh R. ORCOD ID: 0000-0001-8271-5816; Abdigoudarzi M. ORCOD ID: 0000-0002-9239-5972

ABSTRACT

Schistosomiosis, due to *Schistosoma turkestanicum*, is a very important disease. It can cause growth reduction, decrease wool product, reduce meat products and cause weight loss in sheep herds. According to the present records, this disease has spread from the north to the central parts and south of Iran. Stool samples were collected on accidental multiple trials basis from sheep herds, in different seasons of the period of study from 2011 to 2014. The samples were transferred to the laboratory and subjected to direct method and Clayton Lane method. The infection rate was totally 3 percent in the sheep. Different infection rates were observed in seven different places of Dasht Arjan. This rate was from 2 percent in Khane Zenian to 11.2 percent in Chehel Cheshmeh. The minimum number of counted eggs in 1 gram of stool was 1 egg and the maximum counted number of eggs was 6 eggs in 1 gram of stool sample. Finally, it should be noted Dashte Arjan is regarded as an infected area and other epizootic data on the presence of intermediate host, and different climatological factors should also be investigated in the future.

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Introduction

Schistosoma turkestanicum, is a species that has been renamed from *Ornithobilharzia turkestanicum* to *S. turkestanicum*. In a study (Abdulssalam and Sarwar, 1952) *Ornithobilarizia* spp was studied by ITS2 and ITS1 molecular markers collected from China and finally it was determined that this genus belonged to Schistosoma; therefore, it can be concluded that this species is from *Schistosoma (Ornithobilarizia)* genus and *Schistosomatidae* family (Abdulssalam and Sarwar, 1952). This parasite lives in the mesenteric vessels, portal vein of liver, lung and other blood vessels of sheep, goats, cattle, buffaloes, donkeys, camels, mule and boars (Ale-Dawood, 1963; Al-Toma, 2011; Arfa et al., 1965; Eslami, 1998). *S. turkestanicum* was first found in the cattle from Russian Turkestan and named *S. turkestanicum* (Eslami et al., 1998). The diagnosis of *S. turkestanicum* is troublesome. The observation of parasite eggs in stool sample is

*Corresponding Author: Gholemreza Karimi
E-mail:karimighr2003@yahoo.com

going the confirm the infection, but the number of eggs in stool sample is very few. This is the reason why many workers have recourse to serological tests (Geng, 1994).

This parasite has been reported in Southern Russia, China, Mongolia, Turkestan (Kazakhstan, Kyrgyzstan, Turkmenistan, Uzbekistan), Pakistan, India, Iraq, Turkey and Iran (Ghadirian and Hoghooghi, 1973; Hosseini et al., 1997; Karimi, 2003) and it causes economic loss from the viewpoint of decreasing different kinds of loss in products from livestock (i.e. decrease in growth, wool, meat and sheep weight) Liver cirrhosis, bile ducts degeneration, blood vessel stenosis are some of the common pathological findings in *Schistosomiosis* (Karimi et al., 2003). There are documented reports of *S. turkestanicum*, in sheep herds from Babolsar (Ghadirian and Hoghooghi, 1973), Isfahan (Karimi et al., 2014), Khouzestan (Machattie, 1936; Maleki et al., 1994). Some epizootical events were recorded from Eghlid (Fars) (Mansourian, 1995) and some parts of Mazandaran province (Ghadirian and Hoghooghi, 1973). Treatment of infected sheep with Praziquantel and Tricholorphen were unsuccessful, and no significant changes were observed in egg count before and after treatment (Massoud, 1973).

Regarding above comments and importance of *S. turkestanicum*, stool samples were collected from different part of Shiraz region. Direct method and floatation method were applied for samples, then the rate and distribution of infection was estimated and recorded.

Materials and methods

Past studies on the prevalence of *Schistosomiosis* indicated 8 to 15 percent of infection rate. Then, the number of samples needed for this study was estimated using the formula (N =). Regarding P= 0.1, d= 0.03 and 95% confident, the number of samples needed in this study is equal to 384 (n = 384). In this study, random collection in several time intervals was used (cross sectional). For better analysis and to reduce the effect of cluster sampling, number of samples was increased to 600 samples. These samples were collected two times in a year (spring, summer, fall and winter). Samples were collected from 2012 to 2014. 1200 samples were totally collected.

Shiraz district was divided into 5 geographical levels, (nomadic, south, north, east and west). 120 samples were regarded for each level.

Six villages were selected in each level; twenty sheep were selected for sample collection in each village. Nomadic herds were selected for the collection of 120 samples, and totally 1200 samples were collected. Stool samples were collected directly from rectum using a pair of sanitary gloves. Stools were kept in covered vessels. Data regarding, date of collection, place and sheep identification (age, sex, herd owner) were noted on labels on the covers and recorded in a data sheet. Samples were transferred immediately to the laboratory (near the ice packs) and subjected to flotation method (Clayton lane method) (Zinc Chloride) ($ZnCl_2$) was used for the flotation of *S. turkestanicum* eggs. The specific gravity of the solution was 1.20.

A parallel confirmative study was conducted on 30 sheep carcasses inspected in Shiraz abattoir in two different periods of times (during the time of this study). All internal parts of sheep bodies including liver, lungs, heart, kidneys, pancreas, spleen and mesenteric regions were inspected at the laboratory for the presence of adult parasite (*S. turkestanicum*).

Results

Totally, seven places located in Dashte Arjan including (Chehel Cheshmeh, Naeem Abad, Zeloa, Shilan, Nomade herds, Cheramakan, Khaneh Zanian were experimentally found to be positive regarding the presence of eggs of *S. turkestanicum* in collected samples (Figure 1 and Figure 2). Totally three percent of sheep were estimated to be infected with *S. turkestanicum* in Shiraz district. The rate of infection for positive recorded places were, 11.2 %, 10%, 8.4%, 6.7%, 5% , 4.8% and 2% respectively for above-mentioned regions (Table).

The result of the confirmative study showed there were no adult worms in 30 carcasses inspected in Fars (Shiraz) abattoir at the time of study (collection time of study).

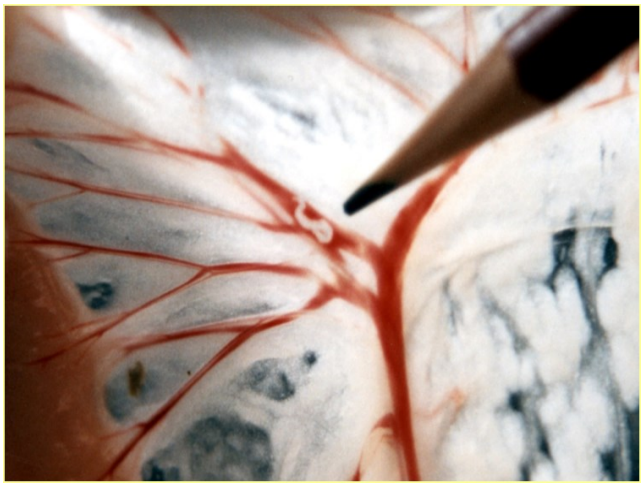

Figure 1. Original, adult stage of *Schistosoma turkestanicum* in mesenteric vessel.

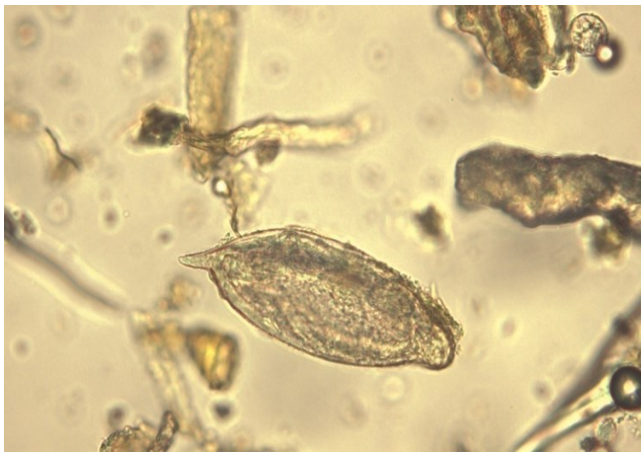

Figure 2. Original, egg of Schistosoma turkestanicum prepared by floatation method

Table 1. Different infection rate of *Schistosoma turkestanicum* in seven places in Shiraz region

Geographical places	Collected samples	Percent of positive cases
Naeem Abad	20	10
Khaneh Zanian	20	2
Chehel Cheshmeh	20	11.2
Shilan	20	6.7
Zeloa	20	8.4
Cheramakan	20	4.8
Nomade herds	20	5
Other places	1060	0
Shiraz region	1200	3

Discussion

In a pilot project research by Ale -Dawood on 1963 the infection from Dezful was recorded (Massoud, 1974). In a study by Arfa (1965) *S. turkestanicum* infection rate in sheep (28%) was reported from Dezful north of Khuzestan province for the first time, (Sahba and Malek, 1976). Maleki et al. (1994) in a pathological study on 1994 recorded the infection from Fars region, but did not regard the nomadic herds (Skerman and Hilard, 1967). In our study the 5 percent infection rate was calculated on stool samples collected from nomadic herds. Maleki et al. (1994) tissue samples were collected post-mortem from intestine, mesenteric lymphatic nodules and liver (Skerman and Hilard, 1967). The place of study was northern part of Fars (Eghlid), but the present study was done in the southern part of Fars (Dashte Arjan).

In other research on sheep herds from Southern part of Khouzestan province (Shadgan region) 13.4 percent of sheep were positive for *S. turkestanicum* (Skrjabin, 1913).

Schistosoma turkestanicum has been reported from different parts of Iran including; Khouzestan (Maleki et al., 1994; Massoud, 1974; Soulsby, 1982), Isfahan (Karimi et al., 2014), Babolsar (Massoud, 1973), Juybar (Ale-Dawood, 1963) and Fars (Eghlid) (Mansourian, 1995). Some of them were observed as epizootic event. In an epizootic case from Babolsar, [September up to the end of winter (March)], there were 60 to 80 percent of sheep which died from five herds each with 150 sheep. In a study on sheep from Babolsar, there were 72 percent positive stool samples on the basis of finding eggs of S. turkestanicum (Ale-Dawood, 1963; Massoud, 1973).

In a study by Eslami et al. (1998) in Mazandaran (Juybar) from September 1998, 17.5 percent of herds and 7.6 percent of sheep were found to be positive.

In a recent study in Mazandaran region 15 percent of sheep were reported positive for *S. turkestanicum* (Urquhart, 1987).

Iran plateau is divided to four zoological zones (Wang et al., 2009).

The *S. turkestanicum* infection has been reported from three zones (including, northern part of Alborz Mountains, central desert from western Azarbaijan to Khorasan and Persian Gulf to Tigris (Dejleh) river. There are no sheep growing in central desert of Iran, so no infection is recorded (Ale-Dawood, 1963; Yamaguti, 1958). Research studies on snails of Iran showed that Lymnaea gedrosiana is the intermediate host of this parasite (Maleki et al., 1994). These snails have been collected from different parts of Iran except Lorestan province (Maleki et al., 1994; Wang et al., 2009; Yamaguti, 1958). There are a lot of water sources and marsh-land, where sheep are kept and graze around these water sources and they could be easily infected to *S. turkestanicum*.
In this study different symptoms of sub-clinical form of infection such as harsh hair, weakness, anemia, hydrothorax, ascites, diarrhea and wool loss were seen in infected animals.

Totally three percent of sheep were estimated to be infected with *S. turkestanicum* in Shiraz region (Dashte Arjan). Regarding previous studies there is no suitable anti-parasite drug available and snail control could not be achieved when there may be different ecological problems. Finally, different responsible sectors are to be aware of the presence of infection to provide suitable measures for infection control.

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References

Abdulssalam, M., & Sarwar, M. M. (1952). *Occurrence of ornithobilharzia turkestanicum in Pakistan.* Paper presented at the 4th Pakistan Scientific Conference, Pakistan.

Ale-Dawood, M. (1963). *Dezful bilharziosis pilot project (3rd quarterly report).* Tehran, Iran: Scool of public Helth and Reaserch Institute of Hygiene.

Al-Toma, Z. (2011). The liver cirrhosis caused by Ornithobilharzia turkestanicum in Basra governorate. *Journal of College of Education, 1* (5), 61-88.

Arfa, F, Sabbaghian, H., & Ale-Dawood, H. (1965). Studies on Ornithobilharzia turkestanicum. *Annual Parasitology & Human Competetive*, 40, 45-50.

Eslami, A. (1998). Veterinary Helminthology 2th ed. (Trematoda), Tehran, Iran: University of Tehran.

Eslami, A, Sarafrazi, M, & Tabatabaee, A. H. (1998). Current status of ovine Ornithobilharzia turkestanicum infection in Juybar, Mazandaran. *Pajuhesh and Sazandegh, 10*(35), 106-107.

Geng, J. (1994). Preliminary studies on the diagnosis of cattle Ornithobilharziosis by ELISA, *Journal of Jilin Agricultural University, 16*(3), 88-91.

Ghadirian, E., & Hoghooghi, N. (1973). The presence of snails of veterinary importance in Isfahan. Iran. *British Veterinary Journal,* 129, 1-3.

Hosseini, S. H, Eslami, A, & Hadadzadeh, H. Epiolemiology of Ornithobilharzia (1997). turkestanicum in sheep in Babolsar (Mazandaran), Iran. *Journal of the faculty of veterinary Medicine, University of Tehran, 52*(3), 53-59.

Karimi, G. (2003). Epidemiological studies on Ornithobilharzia turkestanicum in sheep and its snail in Shadegan area and evaluation of serological tests for diagnosis of infection. *PhD Thesis,* Ahwaz University, Khuzestan, Iran.

Karimi, G., Akhavizadeghan, M. A, Derakhshanfar, M., & Paykari, H. (2003). A survey on infection rate of Ornithobilharzia turkestanicum in sheep in Shadeghan. *Journal of the Factutly of Veterinary Medicine, University of Tehran, 61*(4), 325-329.

Karimi, G., Paykari, H, Motamedi, G., Abdigoudarzi, M, Rivaz, S, & Alamian, S. (2014). A survey on Ornithobilharzia turkestanicum (Skrjabin, 1913) Infection in sheep of Mazandaran province, Iran, using flotation method. Pajuhesh and Sazandeghi, 27(103), 31-36.

Machattie, C. (1936). A Preliminary note on the life history of Schistosoma turkestanicum skrjabin (1913). Transaction of the *Royal Society of Tropical Medicine and Hygiene,* 30, 115-128.

Maleki, M., Khodakaram, Tafti, A., Oryan, A., Aslani, M. (1994). Macroscopic and microscopic finding of ornithobilharziosis in local nomadic herds of sheep and gouts in fars provins, *Pajouhesh and Sazandgi, 7*(24), 66-70.

Mansourian, A. (1993). Fresh water snails fauna of Iran. *PhD Thesis,* Tehran Medical Sciences University, Tehran, Iran.

Massoud, J. (1973). Studies on the Schistosomes of domestic animals in Iran. I. Observation on the Ornithobilharzia turkestanicum (skrjabin, 1913) in Khoozestan. *Journal of Helminthology* 47(2), 165-180.

Massoud, J. (1974). The effect of variation in miracidial exposure dose on laboratory infection of Ornithobilharzia turkestanicum in Lymnaea gedrosiana. *Journal of Helminthes*, 48, 139-144.

Sahba, G., & Malek, E. A. (1976). Dermatitis caused by cercariae of O .turkestanicum in the Caspian sea area of Iran . *American Journal Tropical Medicine and hygiene*, 28, 912-913.

Skerman, K, D, & Hilard, J, J. (1967). *A Handbook for studies of Helminth Parasites of Ruminants.* Tehran, Iran: Near East Animal Health institute.

Skrjabin, K. (1913). Schistosoma turkestanicum, non. ap. ein. neuri. parasitol. de rinde. Aus Russisch Turkestan, zeit. F. *Infektions Krankheit*, 13, 451-468.

Soulsby, E. J. L. (1982). *Helminths, arthropods and arotozoa of domesticated animals. 7th ed*, , London, UK: Bailliere Tindall.

Urquhart, G. M., Armour, J., Duncan, J. M., Jennings, F. W. (1987). *Veterinary Parasitology.* , London, England: Longman.

Wang, C. R., Li, L., Ni, H. B., Zhai, Y. Q., Chen, A. H., Chen, J., Zhu, X. Q. (2009). Orientobilharzia turkestanicum is a member of Schistosoma genus based on phylogenetic analysis using ribosomal DNA sequences. *Experimental Parasitology, 121*(2), 193-197.

Yamaguti, S. (1958), *Systema helminthum, the (digenea) trematodes of vertebrates.* Okayama Japan: University of Medical School.