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Research Article

Investigation of Lipid Profile and Liver Enzymes in Rats Fed on Trans Fats Obtained from Cotton Oil

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ABSTRACT

It is known that the intake of trans fats, commonly used in the food industry around the world, has a negative effect on healthy and balanced nutrition. The aim of this study was to determine the biochemical changes in an organism following the consumption of cotton oil (CO) or trans-fat obtained from cotton oil (TF). A total of 18 Wistar Albino male rats were used. The research was performed in SUDAM unit. Rats divided into 3 equal groups: The control (C) group was fed with a standard rat diet, the CO group was given the standard diet added with 5% CO, and the TF group was given the standard diet added with 5% TF (including 30% trans-fat). *Ad libitum* feeding and water were allowed for 8 weeks of the experimental period. Blood samples were collected on day 0 by the supraorbital method and on day 60 by the intracardiac method under anesthesia. Glucose, HbA1c, triglyceride, total cholesterol, HDL- cholesterol, LDL- cholesterol levels, AST, ALT, ALP, GGT, and SOD activities analyses were performed. SPSS (19.0) program was used for the statistical analyses. Trans fat feeding increased LDL-cholesterol and triglyceride levels, decreased HDL-cholesterol level, and caused some changes in the activities of the liver enzymes investigated.

Keywords: Cotton oil, lipid profile, liver enzymes, rat, trans fat

Pamuk Yağından Elde Edilen Trans Yağlarla Beslenen Ratlarda Lipid Profili ve Karaciğer Enzimlerinin Araştırılması

ÖZET

Sunulan çalışmada, trans ve pamuk yağlarının organizmada meydana getirdiği biyokimyasal değişikliklerin belirlenmesi amaçlandı. Çalışmada 18 adet 12 haftalık Wistar Albino cinsi erkek rat kullanıldı. Ratlar 1. Grup kontrol, diğer 2 grup çalışma grubu olarak toplam 3 gruba ayrıldı. Sekiz hafta süresince 1. Gruba standart rat yemi ve su, 2. Gruba pamuk yağlı yem ve su, 3. Gruba trans yağlı yem ve su *ad libitum* olarak verildi. Çalışma gruplarından 0. günde supraorbital ve 60. günde ise intrakardiyak yöntemle anestezi altında kan alımı gerçekleştirildi. Ratlardan alınan kan örneklerinden glikoz, HbA1c, trigliserit, kolesterol, HDL, LDL, AST, ALT, ALP, SOD ve GGT analizleri yapıldı. Analizler için SPSS programı kullanıldı. Sunulan çalışmada trans yağların, LDL ve trigliserit konsantrasyonunu arttırdığı, HDL konsantrasyonunu azalttığı, bazı karaciğer enzim değerlerinde değişmelere neden olduğu görülmüştür. Trigliserit, LDL, ALP analizlerinde istatistiksel açıdan anlamlı sonuçlar elde edilmiştir ($p<0.05$). GGT ve AST analizlerinde ise istatistiksel açıdan anlamlı sonuçlar elde edilememiştir ($p>0.05$).

Anahtar Kelimeler: Pamuk yağı, lipid profili, karaciğer enzimleri, rat, trans yağ

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Introduction

All living organisms need to be fed sufficiently and balanced in order to maintain their vital functions (Çelebi and Karaca, 2006). The nutrients necessary for maintenance and vital activities are stored in the body. The most important of these nutrients; carbohydrates, proteins, and fats. Energy for the digestion of these nutrients is provided by the organism. The organism takes most of this energy from the fats. The fats are an important part of the cell membranes, involve in the absorption of fat-soluble vitamins, and are the source of essential fatty acids in the body (Karaali, 1997; Murray et al., 2003). Depending on the double bonds in their structure, the fatty acids are classified as saturated or unsaturated (Dutton, 1979). Unsaturated fatty acids are either at cis or trans form depending on the location of the hydrogen atoms on the double bonds (Dutton, 1979). Cis fat is found abundantly in nature while trans fat is present in industrial fats. Trans fat forms when cis unsaturated fats are hydrogenated to remove the double bonds and become saturated fats. Presence of saturated or unsaturated fatty acids in the composition of the fats directly affects the cholesterol levels in the blood; saturated fatty acids increase the level while unsaturated fatty acids decrease it.

Recent studies have demonstrated that trans fatty acids increase cholesterol levels similarly like saturated fatty acids (Sanders et al., 2000; Lichtenstein et al., 2003; Wijendran et al., 2003; Murray et al., 2004). Trans fatty acids raise low density lipoprotein (LDL) levels and reduce high density lipoprotein (HDL) levels. Furthermore, by elevating the LDL/HDL-cholesterol ratio they also increase the risk for heart diseases (Kayahan, 2003; Tasan et al., 2007). They also play critical roles in obesity, cancer, Alzheimer, diabetes, allergy, and fetal development (Innis et al., 1999; Kıralan et al., 2005; Kavanagh et al., 2007). Due to the changes in liver enzymes negative effects of the trans fatty acids are observed in the liver, muscle tissue, bones, and joints such as liver and muscle tissue damage, bone resorption and degeneration (Sharma et al., 2012). Increased intake of trans fatty acids by humans leads to an increase in the number of deaths due to heart attacks. Industrially produced trans fatty acids are effective in increasing this number.

Additionally, the negative effects of trans fatty acids on type 2 diabetes can be observed (Kıralan et al., 2005). Feeding the children during the developmental ages with trans fatty acid containing nutrients have increased the frequency of asthma and allergic disorders (Stender and Dyerberg, 2004). It has been observed that the use of trans fatty acids in pregnant women adversely affects the development of the fetus and increases the number of premature births. Trans fatty acid containing feeding can also be said to increase the probability of Alzheimer's disease in people of middle ages and older (Kıralan et al., 2005).

In this study, the effects of feeding male rats with either cotton oil or trans fat obtained from cotton oil were investigated on blood glucose, HbA1c, triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol levels, AST, ALT, ALP, GGT, and SOD activities and live weights.

Material and Methods

For this study, 18 Wistar Albino male rats that 12 weeks old, weighing 350-380 g were used as animal material. Rats were housed in rooms with heat and light adjustment. Feed and water were given as *ad libitum* for 8 weeks of the experimental period. The rats were divided into 3 groups as follows:

1. Group (Control Group, C, n = 6): Standart rat diet

2. Group (Cotton Oil Group, CO, n = 6): Standart rat diet + 5% cotton oil

3. Group (Trans Fat Grovup, TF, n = 6): Standart rat diet + 5% trans fat obtained from cotton oil (contains 30% trans fat)

Preparation of diets:

Cotton oil diet: 95 g standard diet was ground to powder and 5 g cotton oil was added. Then, pellets were made and kept at - 20˚C.

Trans fat diet: 95 g standard diet was ground to powder and 5 g trans fat obtained from cotton oil was added. Added fat contained 30% trans fat in 5 g. Then, pellets were made and kept at - 20˚C

Blood samples were collected on day 0 by the supraorbital method and on day 60 by the intracardiac method under anesthesia. Analysis of glucose, triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, AST, ALT, ALP, and GGT was performed in biochemical autoanalyzer (Architect ci 8200 Abbott). HbA1c was measured with Premier HB9210. SOD analysis was performed on the ELISA reader (Biotek ELX800) with the Cayman Chemical branded catalog number 706002.

Data were analyzed with one-way ANOVA and t-test. In comparison between day 0 and day 60 independent t-test was used and Duncan test was employed to detect differences among the study groups. The means and standard errors were given and $P < 0.05$ was considered as significant. All the statistical analyses were performed with the SPSS program.

This study was performed at the Selcuk University Experimental and Medical Center (SUDAM). The ethical approval (2017/28) was obtained from the Ethical Committee of the Faculty of Veterinary Medicine, Selcuk University. It was funded by SUBAP project number 17202049.

Results

The results for biochemical parameters and live weights on day 0 and 60 in the study groups were given in Table 1.

There was no statistically significant difference between the study groups and the control group in the glucose levels on day 60 ($P > 0.05$). HbA1C values in the blood samples were found to be statistically significant in CO and TF groups compared to the C group on day 60 ($P < 0.05$). Triglyceride value was significantly lower on day 60 in the CO group compared to C and TF groups ($P < 0.05$). Cholesterol and HDL-cholesterol levels did not differ among the groups on day 60 while the LDL-cholesterol level showed significant differences among the groups both on day 0 and day 60. As GGT, AST and ALT activities were compared among the groups, no statistically significant difference was observed on day 60 ($P > 0.05$). The ALP activity was found to be significantly higher on day 60 in the TF group compared to C and CO groups ($P < 0.05$). Superoxide dismutase activity was higher on day 60 in the TF group ($P < 0.05$). When the live weight values of the control group and the study groups were compared, it was observed that the live weight values of the CO and TF groups were statistically significantly lower on day 60 compared to the control group ($P < 0.05$).

Discussion and Conclusion

In this age of the world, many health problems are caused by irregular, unbalanced and inadequate nutrition. Most of the daily diets include hydrogenated margarine, fast foods, and

Table 1 The results for the parameters investigated on day 0 and 60 of the experimental period (mean ± SE) (n=18)

	DAY	n	CONTROL (C)	n	COTTON OIL (CO)	n	TRANS FAT (TF)
Glucose (mg/dl)	0.	6	192.33 ± 7.82 $^{a.A}$	6	178.33 ± 3.70 $^{a.A}$	6	181.66 ± 9.61 $^{a.A}$
	60.	6	262.83 ± 18.96 $^{a.B}$	6	232.16 ± 26.02 $^{a.A}$	6	235.16 ± 11.49 $^{a.B}$
HbA1c (%)	0.	6	4.41 ± 0.04 $^{a.A}$	6	4.28 ± 0.09 $^{a.A}$	6	4.43 ± 0.05 $^{a.A}$
	60.	6	5.31 ± 0.08 $^{a.B}$	6	4.93 ± 0.14$^{b.B}$	6	4.76 ± 0.08 $^{b.B}$
Triglyceride (mg/dl)	0.	6	117.33 ± 14.87 $^{a.A}$	6	103.33 ± 25.24 $^{a.A}$	6	128.16 ± 17.79 $^{a.A}$
	60.	6	190.66 ± 34.62 $^{a.A}$	6	108.16 ± 14.91$^{b.A}$	6	230.50 ± 27.43 $^{a.B}$
Total Cholesterol (mg/dl)	0.	6	75.16 ± 2.93 $^{a.A}$	6	71.66 ± 3.01 $^{a.A}$	6	74.00 ± 1.86 $^{a.A}$
	60.	6	66.16 ± 3.70 $^{a.A}$	6	69.00 ± 3.18 $^{a.A}$	6	65.50 ± 2.66 $^{a.B}$
HDL-cholesterol (mg/dl)	0.	6	51.50 ± 2.62 $^{a.A}$	6	49.66 ± 3.27 $^{a.A}$	6	54.00 ± 1.50 $^{a.A}$
	60.	6	49.00 ± 2.51 $^{a.A}$	6	53.50 ± 3.28$^{a.A}$	6	46.50 ± 2.44 $^{a.B}$
LDL-cholesterol (mg/dl)	0.	6	16.00 ± 0.89 $^{a.A}$	6	15.16 ± 0.87 $^{ab.\ A}$	6	12.83 ± 0.87 $^{b.A}$
	60.	6	13.00 ± 0.85$^{b.B}$	6	16.00 ± 1.09 $^{a.A}$	6	13.50 ± 0.56 $^{ab.\ A}$
AST (U/L)	0.	6	91.83 ± 3.65 $^{a.A}$	6	85.50 ± 4.95 $^{ab.\ A}$	6	72.66 ± 6.08 $^{b.A}$
	60.	6	131.83 ± 41.37 $^{a.A}$	6	134.83 ± 41.99 $^{a.A}$	6	72.66 ± 7.08 $^{a.A}$
ALT (U/L)	0.	6	48.66 ± 2.67 $^{a.A}$	6	49.66 ± 2.90 $^{a.A}$	6	53.66 ± 4.18 $^{a.A}$
	60.	6	94.66 ± 21.71 $^{a.A}$	6	96.83 ± 39.39 $^{a.A}$	6	54.50 ± 1.74 $^{a.A}$
ALP (U/L)	0.	6	211.00 ± 40.37 $^{a.A}$	6	266.00 ± 24.76 $^{a.A}$	6	291.16 ± 29.84 $^{a.A}$
	60.	6	199.66 ± 28.38 $^{b.A}$	6	284.66 ± 32.03 $^{b.A}$	6	390.33 ± 35.13 $^{a.B}$
GGT (U/L)	0.	6	1.35 ± 0.17 $^{a.A}$	6	1.35 ± 0.17 $^{a.A}$	6	1.48 ± 0.13 $^{a.A}$
	60.	6	0.81 ± 0.29 $^{a.A}$	6	1.21 ± 0.18 $^{a.A}$	6	1.26 ± 0.20 $^{a.A}$
SOD (U/ml)	0.	6	0.081 ± 0.007 $^{a.A}$	6	0.081 ± 0.007 $^{a.A}$	6	0.076 ± 0.003 $^{a.A}$
	60.	6	0.100 ± 0.008 $^{b.B}$	6	0.095 ± 0.009 $^{b.A}$	6	0.227 ± 0.055 $^{a.B}$
Live Weight (g)	0.	6	374.66 ± 10.09 $^{a.A}$	6	353.00 ± 9.86 $^{a.A}$	6	368.50 ± 5.64 $^{a.A}$
	60.	6	490.00 ± 12.20 $^{a.B}$	6	441.66 ± 14.45$^{b.B}$	6	443.66 ± 8.20 $^{b.B}$

For each parameter, differences were indicated in the same line with a, b, c.(ANOVA-Duncan) and in the same column with A, B, C (Paired t test).

shortening. Oils used daily for nutrition contains a high rate of trans fats. The negative effects of trans fats on human health are mentioned in many studies. (Gürcan, 2002; Lichtenstein et al., 2003; Lemaitre et al., 2006). Therefore, this study aimed to investigate some biochemical parameters and live weight gains in the male rats which were fed diets supplemented with cotton oil or trans fat obtained from cotton oil.

There was no statistically significant difference in the glucose levels between groups ($P > 0.05$). When the comparison was made between days within the groups, it was observed that the glucose levels of the TF group on day 60 were statistically higher than day 0 ($P < 0.05$). Kavanagh et al. (2007) evaluated the effects of trans fats on obesity and insulin sensitivity. In contrast to our findings, trans fats were found to have no significant effect on glucose levels compared to the control group. In addition, Destaillats et al. (2005) indicated that decreasing the amount of trans fats in foods had no potential benefit on glucose balance. Huang et al. (2009) showed in a study performed in rats that the trans fat fed rats and control rats had a similar increase in the blood glucose levels. This was thought to be due to the standard rat feed like in the present study. A similar but not significant increase in the blood glucose level between day 0 and day 60 was also observed in this study in the CO group. The present results are in accordance with those reported by Huang et al. (2009) and could indicate that, the standard rat diet could have caused the increase in the blood glucose levels regardless of the fat feeding.

In the present study, CO group rats had significantly lower triglyceride level compared to other groups on day 60; however, TF group rats had significantly higher triglyceride levels on day 60 compared to their day 0 levels ($P < 0.05$). Some earlier studies using trans fat diets observed that triglyceride concentration increased in a similar way as the values presented in the study (Zock and Katan, 1992; Sundram et al., 1997; Rivellese et al., 2003) while others reported no differences in triglyceride levels due to trans fat feeding (Almendingen, 1995; Roos et al., 2003). Moreover, Peter et al. (1992) stated that trans fat taken with foods did not cause major changes in the triglyceride

levels of the blood and the differences could be due to fat supplementation to the feeds.

When the cholesterol levels were examined, there was no statistically significant difference in the cholesterol levels among the groups ($P > 0.05$). There was no statistically significant difference in the LDL-cholesterol levels between groups ($P > 0.05$). The HDL-cholesterol levels did not differ among the groups on day 60 ($P > 0.05$). Earlier studies have demonstrated that trans fat consumption lowers the HDL-cholesterol levels and increases the LDL-cholesterol and total cholesterol levels (Zock and Katan, 1992; Sundram et al., 1997; Rivellese et al., 2003). Murray et al. (2004) reported that nutrients which contain trans fats elevated LDL cholesterol levels, decreased HDL cholesterol levels and increased risk of coronary heart disease. The HDL and LDL-cholesterol values in the study of Murray et al. (2004) are consistent with the results of the present study. Accordingly, it may be considered that changes in cholesterol levels may be observed in a shorter time when the amount of trans fat in nutrients is increased. Also, Peter et al. (1992) stated that trans fat intake decreases HDL/LDL-cholesterol ratio without a significant change in the triglyceride levels. In the present study, the TF group had a decreased HDL/LDL-cholesterol ratio compared to the other groups.

It is stated that when the intake of industrially produced trans fats causes an increase of 2% in the energy levels, the risk of developing heart disease rises by 55% (Stender and Dyerberg, 2004). On the other hand, replacing the source for this energy from trans fats to unsaturated fats decreases the rate of coroner diseases about 53% (Kitao and Hattori, 1983). Feldman (1999) demonstrated that an increase in the total and LDL cholesterol values results in an increase in the risk of developing cardiovascular disease and elevation in the HDL cholesterol causes a decrease in that risk. Mersink and Katan (1990) stated that the intake of trans fats was associated with an increase in the plasma lipid levels. In addition, replacing cis-unsaturated fatty acids in foods with trans fats caused an increase in total and LDL cholesterol levels. Based on the information given above, it is understood that the long period of trans fat feeding as in the current study could increase the risk of developing cardiovascular diseases and that the replacement of trans fats in the diets with unsaturated fats would reduce the incidence of coronary diseases.

In the present study, liver function tests were performed and AST and ALT activities were found be not affected by the experimental feedings although TF group had a numerically, but not significantly, lower values ($P > 0.05$). However, ALP activities were significantly higher in the rats of the TF group ($P < 0.05$). Gama glutamyl transpeptidase activities were also unaffected and based on the report by Ersoy (2012), no changes in the GGT activities could be related to the location of the liver damage. It is thought that GGT activities may significantly change with longer duration of feeding the rats with trans fats.

When HbA1C and body weight values were compared between groups on day 60, HbA1C and body weight values of CO and TF groups were found to be statistically lower than the control group ($P < 0.05$). Kavanagh et al. (2007) fed monkeys for 6 years with trans fats containing diets which caused a 7.2% increase in the body weight while feeding with a monounsaturated fat diet resulted in a 1.8% increase. In addition, a study with women (Stender and Dyerberg, 2004) showed that the increase in the risk of obesity leads to an increase in the likelihood of type 2 diabetes in humans. More studies (Stender and Dyerberg, 2004; Kıralan et al., 2005) indicate that trans fats facilitate the development of type II diabetes. Kırılan et al. (2005) reported that trans fats caused an increase in insulin resistance by causing changes in the ion structure of the cell wall. In another study (FDA, 2003), it was stated that trans fats caused undesirable effects in diabetic patients by reducing the response of red blood cells to insulin, and a positive correlation between the development of type 2 diabetes and trans fat intake in obese women has been observed. Ghafoorunissa (2008) stated that the increase in the intake of linoleic acid (poliunsturated omega 6) adversely affects insulin resistance and therefore trans fat intake should be reduced.

In recent studies, trans fats are reported to be associated with the development of some types of cancers (Innis et al., 1999), type 2 diabetes, and they trigger asthma and allergies in children (Tasan and Daglioglu, 2005; Tasan et al., 2007). Therefore, developed countries impose restrictions on the amount of trans fats in nutrients. FDA and WHO inform the public about reducing the consumption of trans-fat-containing foods. Countries such as the USA, Netherlands, and Canada require that the labels on the food products should include information about their trans fat contents (FDA, 2003; Stender and Dyerberg, 2004).

In Turkey, efforts are made to decrease the use and consumption of trans fats. Restrictions are considered to limit the use of industrial trans fatty acids in food products. Specifically, the use of alternative methods for the hydrogenation process is suggested in the production of margarine and shortenings. New regulations have been applied for the preservation and storage of foods. By providing public awareness by the public and non-governmental organizations, it is aimed to reduce trans fatty acid consumption.

In conclusion, the presence of trans fats in foods causes significant changes in biochemical blood parameters, which leads to the metabolic disorders and therefore the use of trans fats should be taken into consideration in order for a healthy life.

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Research Article

Prevalence of Gastrointestinal Parasites in Stray Cats of İzmir

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ABSTRACT

Gastrointestinal parasites of cats can affect animal health and welfare, as well as human health because of some zoonotic parasites including *Toxoplasma gondii*, *Cryptosporidium* spp., *Isospora* spp., *Blastocystis* sp., and *Toxocara* spp. Therefore, it is fairly important to investigate the presence of gastrointestinal parasites in stray cats in order to reveal the frequency of parasite diseases and to prevent the spread of parasitic diseases. A total of 465 feces samples were collected from Veterinary Clinics located in 5 different districts of İzmir. For microscopic examination, all feces samples were processed by centrifugation-sucrose flotation. In addition, cat feces with diarrhea were stained by the by Kinyoun acid-fast staining for the diagnosis of *Cryptosporidium* spp. As a result, 73 of 465 (15.6%) cats were found to be infected with at least one of the following parasites: *Blastocystis* sp., *Isospora* spp., *Cryptosporidium* spp., *Toxoplasma gondii*-like oocyte, *Toxocara* spp., *Hymenolepis* spp. and *Dipylidium caninum*. Among the studied stray cats, *Blastocystis* sp. was detected as the most prevalent protozoon parasite (10.5%) in stray cats. Overall, the results show that stray cats are a significant source for distribution of various parasite diseases to humans and animals in İzmir, Turkey.

Keywords: Blastocystis sp., Cryptosporidium spp., İzmir, stray cats, Toxocara spp.

İzmir İlinde Sokak Kedilerinde Gastrointestinal Parazitlerin Prevalansı

ÖZET

Kedilerde gastrointestinal parazitler hayvan sağlığını ve refahını etkilemesinin yanında *Toxoplasma gondii, Cryptosporidium spp., Isospora spp., Blastocystis sp., Toxocara spp*. gibi bazı zoonozlar nedeniyle insan sağlığını da etkilemektedir. Bu nedenle, sokak kedilerinde gastrointestinal parazitlerin varlığının araştırılması hastalıkların sıklığını ortaya çıkarmak ve yayılmasını önlemek için oldukça önemlidir. Bu çalışmada İzmir ilindeki sokak kedilerinde gastrointestinal parazitlerin sıklığının dışkı örneklerinin direkt mikroskobik incelenmesi ile belirlenmesi amaçlanmıştır. İzmir'in 5 farklı ilçesinde bulunan veteriner kliniklerine getirilen toplam 465 dışkı örneği toplanmıştır. Mikroskobik inceleme için, tüm dışkı örneklerine santrifüj-sükroz yüzdürme metodu uygulanmıştır. Ayrıca ishalli dışkı örnekleri *Cryptosporidium spp.* varlığının araştırılması amacıyla Kinyoun asit-fast boyama ile boyanmıştır. Sonuç olarak 465 kediden 73'ünün (%15,6*) Blastocystis sp., Isospora spp., Cryptosporidium spp., Toxoplasma* benzeri ookist*, Toxocara spp., Hymenolepis* spp. ve *Dipylidium caninum* türlerinden en az biri ile enfekte olduğu tespit edilmiştir. Sokak kedileri arasında *Blastocystis* sp. en yaygın protozoon paraziti (%10,5) olarak tespit edilmiştir. Sonuç olarak, elde edilen bulgular Türkiye'de İzmir ilindeki sokak kedilerinin çeşitli paraziter hastalıkların insan ve hayvanlara yayılması için önemli bir kaynak olabileceğini göstermektedir.

Anahtar Kelimeler: Blastocystis sp., Cryptosporidium spp., İzmir, sokak kedileri, Toxocara spp.

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Introduction

Humans initiated the cat's domestication process to conserve the grain stores from rodent when they began to cultivate crops and store grain (Clutton-Brock, 1999). Later, because of close contact with domestic cats that has been continued since long years, the relationship known as "human-animal bond" strengthen more and additional benefits such as companionship, socialization, mental health and physical well-being which contribute to development of positive mood in human have been noticed (Deplazes et al., 2011). These positive effects have caused that numerous humans or family living in developing and developed countries have or adopt a domestic cat. For example, it is stated that 34% of families located in United State have domestic cat and nearly half of cat owners consider cats to be family members (Dabritz and Conrad, 2010; Goldstein and Abrahamian, 2015). In addition, many adults or children contact with stray cat in order to feed, play or be friend with them. During this contact, some parasites known as zoonoses can be transmitted from cats to humans since these stray cats are not subjected to a regular anti-parasitic treatment or a routine vaccination program. At the same time, low socio-economic factors and education level, and poor hygiene practices are other important factors which facilitate the transmission of parasites from cats to humans.

Stray animals like cats or dogs are widespread in İzmir which is the third big city of Turkey in respect to population. According to records of Izmir Chamber of Veterinarians, there may be more than 500 thousand stray dogs and stray cats living on the streets of İzmir. These stray animals do not have very well living conditions because of inadequate medical care, even they reach to food which is left by animal lovers. Therefore, insufficient medical care offers negative influences for animal health as well as for human health because of zoonotic diseases. For instance, several types of endo-parasites, including protozoa, cestodes, trematodes and nematodes can infect stray cats and as a result of this, some clinical symptoms like diarrhea, vomiting, anemia, poor growth rate and rarely death can be monitored (Symeonidou et al., 2018). Furthermore, zoonotic diseases such as cryptosporidiosis, toxoplasmosis, giardiasis, blastocystosis, leishmaniasis, toxocariasis, opisthorchiasis, dipylidiasis, and echinococcosis are significant parasitic diseases that also infect humans (Baneth et al., 2016; Goldstein and Abrahamian, 2015).

The frequency of parasites in stray cats living in Turkey is not well known due to lack of sufficient number of studies performed in big cat groups. According to results of available studies, *T. gondii*, *Leishmania infantum* and *L. tropica*, *Toxocara* spp., *Ancylostoma spp*., *Joyeuxiella pasqualei*, *Hydatigera taeniaformis*, *Mesocestoides* spp., *Dipylidium caninum*, *Isospora* spp. and *Cryptosporidium* spp. have been reported in stray cats (Yaman et al., 2006; Can et al., 2014; Öge et al., 2014; Paşa et al., 2015; Can et al., 2016; Korkmaz et al., 2016).

In this study, we aimed to reveal the frequency of gastrointestinal parasites in 465 stray cats living in İzmir province. For this purpose, the presence of gastrointestinal parasites in feces samples collected from stray cats was investigated in concentrated samples by direct microscopy. Moreover, Kinyoun Acid–fast staining method was used for the diagnosis of *Cryptosporidium* spp. This study not only detected the frequency of gastrointestinal parasites but also revealed reservoir potential of stray cats in terms of zoonotic agents harbored.

Material and Methods

Study area and cat population

Between April and October in 2017, a total of 465 feces samples were collected from Veterinary Clinics located in 5 different districts (Çiğli, Karabağlar, Karşıyaka, Konak and Narlıdere) of İzmir. Among the 465 feces samples, 74 was from Çiğli, 25 from Karabağlar, 38 from Karşıyaka, 183 from Konak and 145 from Narlıdere. Of the 465 stray cats, 299 (64.35%) were female and the remaining 166 (35.65%) were male. The protocol for collecting feces from stray cats was declared as exempt according to the instructions and approval of the Institutional Animal Care and Use Committee (IACUC) of Ege University for animal ethical norms (Number: 109/2970).

Microscopic examination

Each feces sample was macroscopically examined to detect the possible presence of cestode proglottids and/or adult nematodes. For microscopic examination, all feces samples were processed by centrifugation-sucrose flotation method as described with minor modifications (Dubey et al., 1970). In brief, feces sample (~10 gr) was first transferred into the 50 ml tube filled with tap water and incubated for two hours at room temperature. Later, tap water was discarded, and 50 ml of sucrose solution (530 g sugar, 1-liter distilled water, 8 ml phenol) was added and emulsified. This mixture was then filtered with two layers of gauze and divided into 15 ml tubes and centrifuged at 400xg for 10 minutes. After centrifugation, approximately 0.5 ml supernatant from the top of each 15 ml tube was collected in another tube and the presence of parasites eggs or oocysts or cysts was investigated between slide-cover slide under light microscopy.

Kinyoun acid -fast staining

Cat feces with (n:50) diarrhea were stained by Kinyoun acid-fast staining for *Cryptosporidium* spp. diagnosis as previously described (Turgay et al., 2012). Briefly, a drop of concentrated feces sample was smeared on a slide, air dried and fixed by methanol. Later, alkaline fuchsin was poured on the slides and incubated for 5 min. Next, the slides were washed with tap water, decolorized by 2.5% sulfuric acid for 1 minute and the slides were stained with 1% methylene blue for 1 min. Finally, slides were washed, air dried and examined under light microscopy using x1000 magnification.

Statistical analysis

Data obtained from this study were processed using PASW Statistics 18. A chi-square test was used to determine the significance between the positivity rates detected in different counties of İzmir and between male and female cats.

Results

Out of 465 feces samples, 73 were found to be infected with at least one parasite (15.6%) (Figure 1). Totally, 61 cats were infected with protozoon and 16 cats were infected with helminth. Among protozoon species, *Blastocystis* sp., *Isospora* spp. and *Cryptosporidium* spp. were identified. Additionally, *Toxoplasma gondii* like oocysts were identified in two feces samples (0.43%). Of the helminths 14 of them were *Toxocara* spp., one of them was *Hymenolepis* spp. and the other was *Dipylidium caninum*. *Blastocystis* sp. was the most identified parasite (10.5%). Percentage positivity value for *Toxocara* spp., *Isospora* spp., *Cryptosporidium* spp., Hymenolepis *spp.* and *D. caninum* were 3.0, 1.0, 1.0, 0.21 and 0.21, respectively. Also, co-infections were found in four cats. Co-infection with *Blastocystis* sp.-*Isospora* spp., *Blastocystis* sp.-*Toxocara* spp., *Blastocystis* sp.-*Cryptosporidium* spp. and *Cryptosporidium*

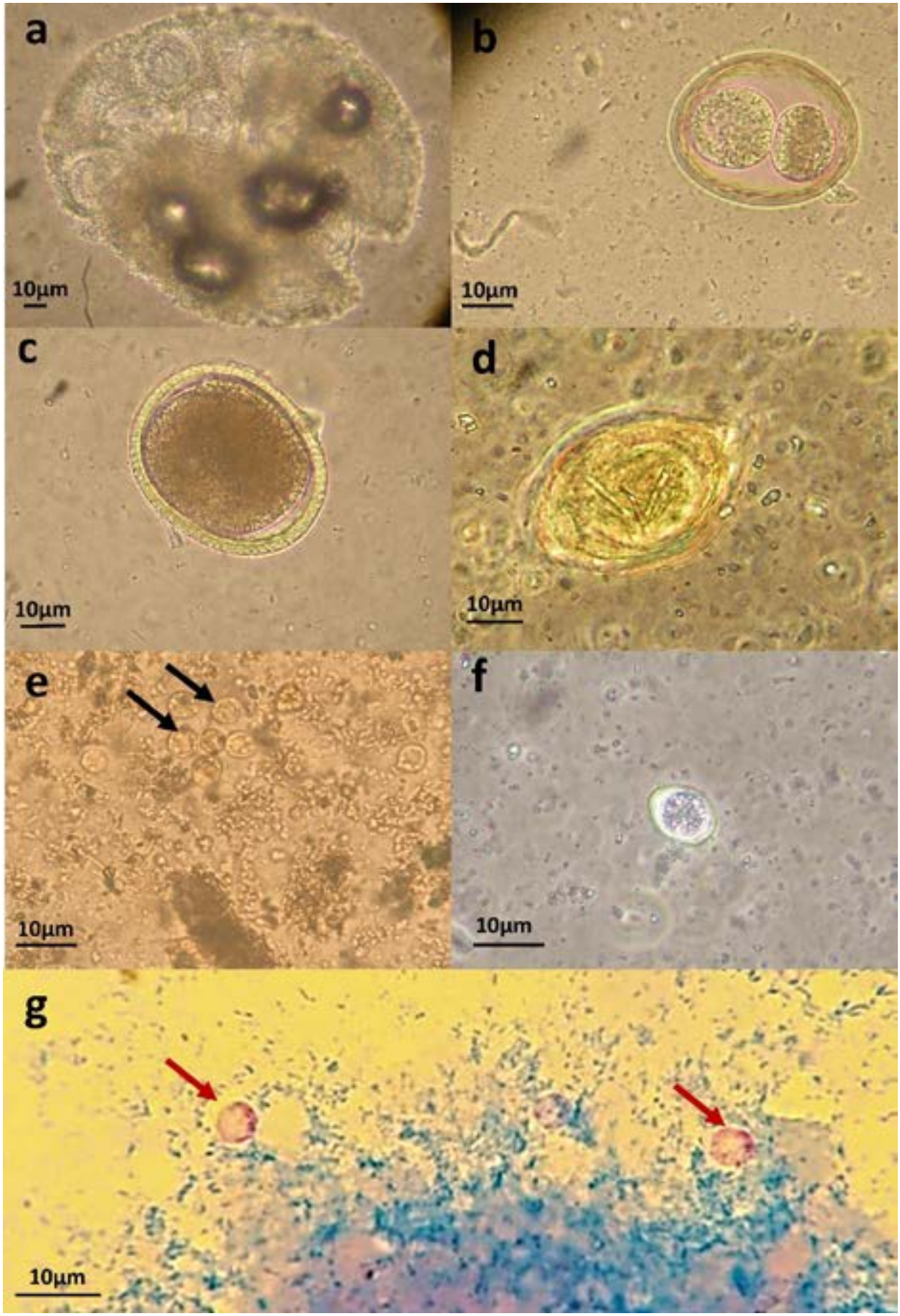

Figure 1. a) Egg packet of *Dipylidium caninum,* **b)** *Isospora spp.* oocyst*,* **c)** *Toxocara* spp. egg**, d)** *Hymenolepis* spp. egg*,* **e)** *Blastocystis* spp. *cysts*, **f)** *T. gondii*-like oocyst, unsporulated**, g)** *Cryptosporidium* spp. oocysts.

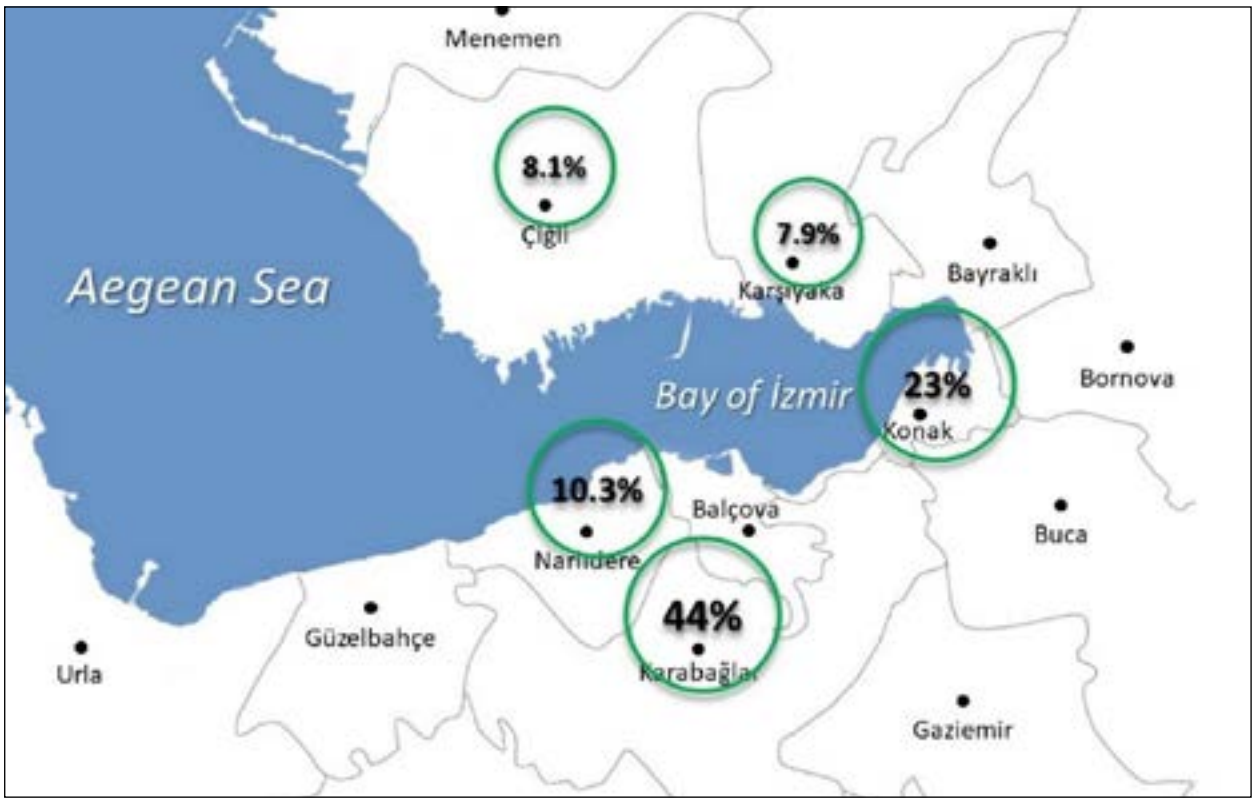

Figure 2. Percentage distribution of positive samples

spp.-*Toxocara* spp, were detected. Karabağlar and Konak had the highest percentage positivity values when compared to other districts (*P<0.05*) (Figure 2). Statistically significant difference was not found when the percentage positivity values were compared among female and male cats (female: 15.38%, male: 14.46%; *P>0.05*). The identified parasites species in each district of İzmir was given in detail in Table 1.

is required to be investigated in specified periods as well as positive animals should be treated with specific drugs.

In this study, we investigated the prevalence of gastrointestinal parasites in a big stray cat group living in İzmir which is third big city of Turkey and found that 15.6% of feces samples were infected with at least one parasite. In Turkey, a study conducted

Table Legends:

Table1. Numbers of the parasite species detected in five different districts of İzmir

			Districts		
Parasite Species	Çiğli	Karabağlar	Karşıyaka	Konak	Narlıdere
Blastocystis sp.	6	5	-	25	13
Toxocara spp.	-	1	1	10	2
Isospora spp.	-	1	-	4	-
Cryptosporidium spp.	-	4	1	-	-
Toxoplasma gondii-like oocysts	-	-	-	2	-
Hymenolepis spp.	-	-	-	1	-
D. caninum	-	-	1	-	-
Total	6	11	3	42	15

* In Narlıdere, co-infection with *Blastocystis* sp.-*Isospora* spp., was detected in one cat. In Konak, co-infection with *Blastocystis* sp.-*Toxocara* spp., *Blastocystis* sp.-*Cryptosporidium* spp. and *Cryptosporidium* spp.-*Toxocara* spp., were detected in three different cats.

Discussion

Considering the importance of one health approach, studies focusing the diagnosis of infectious agents in stray animals are crucial and a lot of studies are being performed in this field worldwide. Although there is numerous stray animal such as dog and cat in Turkey, these animals do not take sufficient medical care, and this threatens animal and human health. Therefore, the presence of infectious agents in these animals

in Kırıkkale province reported that 47% (n=100) of feces samples collected from cats were parasite positive using microscopy. *Isospora spp., Toxocara spp., Joyeuxiella spp., Cryptosporidium spp.,* and hookworm were detected in positive cats. (Korkmaz et al., 2016). In another study performed in Hatay, %87,5 (n=8) of dead cat necroscopy and feces samples was found to be positive for parasites using microscopy. *Toxocara cati, Joyeuxiella pasqualei, Hydatigera taeniaformis, Mesocestoides sp. and Dipylidium caninum* were found in study (Yaman et al.,

2006). It is known that positivity value can change depending on number of samples tested, methods used, geographical region and cat lifestyle.

In our study, *Blastocystis* sp. was detected as the most prevalent protozoon parasite (10.5%) in stray cats although it has not been reported previously in cats of Turkey. In a study from Australia, among the 52 feces samples collected from cats, 67.3% was reported to be *Blastocytis* sp. positive by microscopic examination (Duda et al., 1998). In a different study conducted in US Pacific Northwest, it was found that positivity of *Blastocystis* sp. was 11.65% in 105 feces samples belonging to shelter-resident felines using nested PCR targeting the SSU rDNA fragment. Also, same study reported that none of client-owned cats was positive for *Blastocystis* sp. (Ruaux and Stang, 2014). *Blastocystis* sp. is also accepted as the most common eukaryotic organism detected in gastrointestinal tract of humans (Lepczyńska et al., 2017) and molecular epidemiological investigations have showed that some sub-types of *Blastocystis* sp. play role in zoonotic transmission (Lee et al., 2012; Cian et al., 2017; Greige et al., 2018). Parallel to this, previously conducted studies reported that the frequency of *Blastocystis* sp. was prevalent in humans who live in İzmir province. Results obtained from these studies showed that prevalence of *Blastocystis* sp. was 4.96% in 2005 and increased to 32.33% in 2012 (Değirmenci et al., 2007; Turgay et al., 2012). All these findings indicate that stray cats can be a source of *Blastocystis* sp. transmission to humans in İzmir.

In our study, *Toxocara* spp. was the second prevalent parasite in stray cats of İzmir and was found in 3.0% of (14/465) feces samples. In Turkey, it has been stated that the prevalence of *T. cati* varied from 27.6 to 47.2% in cats (Burgu et al., 1985; Doğanay, 1992; Doğanay and Öge 1993; Öge et al., 2014). A study conducted in Ankara reported that *Toxocara* eggs were found in 13% of feces samples collected from owned cats (Öge et al., 2014). Another study found that the prevalence of *Toxocara* spp. was 48.9% in cats in Kırıkkale (Korkmaz et al., 2016). Worldwide, it has been reported that prevalence of *T. cati* was within range of 8% to 91% in cats (Overgaauw , 1997; Öge and Öge, 2000; Macpherson, 2005; Bowman, 2009; Öge et al., 2014). Although obtained results show different prevalence values, all of them indicate that cats are significant source for the transmission of *Toxocara* spp. to humans.

Cats are definitive host for some apicomplexan parasite such as *Toxoplasma gondii* which can infect all mammalian, including humans as well as birds and cause important clinical cases and economic losses. In this study, three different apicomplexan parasite species were detected in 12 feces samples. Among apicomplexan parasites, *Isospora* spp. and *Cryptosporidium* spp. were detected in five of feces samples tested while *T. gondii*-like oocysts were detected in only two feces samples. In Turkey, the presence of *Cryptosporidium* spp. and *Isospora* spp. has been reported in previously conducted studies on cats. Accordingly, a study showed that *Isospora* spp. and *Cryptosporidium* spp. positivity was found to be 65.9% and 2.12%, respectively (Korkmaz et al., 2016). In two different studies, *Isospora* spp. positivity was reported to be 2.8% and 43% in stray cats (Burgu et al., 1985; Doğanay, 1992). In a different study performed in Turkey, prevalence of *Cryptosporidium* spp. was found to be 13% in kittens (Goz et al., 2005). *T. gondii* oocyst positivity in cat feces samples is lower when compared to antibody positivity because of short term oocyst shedding period. According to this, many studies reported that *T. gondii* oocysts were not detected in seropositive cats (Miró et al., 2014; Dubey et al., 2007; Qian et al., 2012). In our study, *T. gondii* like oocysts positivity was found to be 0.43% in stray cats. For the diagnosis of *T. gondii* infection, further techniques like PCR are needed to be applied to these two positive samples because *T. gondii* oocysts cannot be differentiated from oocysts of *Hammondia hammondi* and *Besnoitia* spp. by microscopic techniques (Dabritz et al., 2007). This condition also demonstrates the importance of molecular tests used in diagnosis of *T. gondii* infection.

In this study, *Hymenolepis* spp. and *D. caninum* were detected in stray cats. Prevalence of these helminths was lower than others. In a study conducted in Elazığ, *D. caninum* positivity in cats was 33% while *Hymenolepis* spp. has not been detected (Altaş et al., 1999). In another study, *D. caninum* positivity in cats was 12.5% and *Hymenolepis* spp. has not been detected (Yaman et al., 2006). In a different study, both *Hymenolepis* spp. and *D. caninum* have not been detected in feces of cats (Korkmaz et al., 2016).

Conclusions

In conclusion, the accumulated data show that stray cats are a significant source for the distribution of various parasites in nature. Therefore, these stray cats contacting with humans should be routinely checked for parasites and cats with parasitic diseases should be treated immediately. By this way, the quality of life of cats can be improved and, transmission of zoonotic parasites to humans can be prevented.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Virological Investigation of Border Disease Infection in Sheep with Abortion Problem

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ABSTRACT

Pestiviruses are important pathogens to economic losses in cattle, sheep, goat and pigs. Border disease virus (BDV) caused by pestiviruses is characterized by low birth weight and congenital disorders in lambs, while infection is subclinical in adult sheep. Transmission of border disease between animals is through direct contact. However, since persistently infected animals are natural sources of the disease, large outbreaks may occur if a persistent infected animal enters the susceptible herd. In this study, the aim was to investigate the presence of BDV by a PCR assay using primers specific for the DNA fragment of the 5 'NC region by taking blood samples from 75 sheep with abortion problems found on Selcuk University Faculty of Veterinary Medicine Internal Medicine Clinic. As a result of the test, all animals were found as negative. Testing of a variety of sample types collected from a larger number of animals is recommended in order to determine the epidemiological existence of border disease.

Keywords: Abortion, Pestivirus, Border disease, PCR

Abort Problemli Koyunlarda Border Disease Enfeksiyonunun Virolojik Olarak İncelenmesi

ÖZET

Pestiviruslar sığır, koyun, keçi ve domuzlarda ekonomik kayıplara yol açan önemli patojenlerdir. Pestivirusların neden olduğu Border Disease Virusu (BDV), kuzularda düşük doğum ağırlığı ve konjenital bozukluklarla karakterize edilirken, yetişkin koyunlarda enfeksiyon subklinik seyretmektedir. Hayvanlar arasında Border hastalığının bulaşması doğrudan temas yoluyla olur. Bununla birlikte, persiste enfekte hayvanlar hastalığın doğal kaynakları olduğundan, persiste enfekte bir hayvan duyarlı sürüye girerse büyük salgınlar meydana gelebilir. Bu çalışmada Selçuk Üniversitesi Veteriner Fakültesi İç Hastalıkları Kliniğine gelen abort problemli 75 koyundan kan örnekleri alınarak, 5 'NC bölgesinin DNA fragmanına özgü primerlerin kullanıldığı PCR testi ile BDV varlığının araştırılması amaçlanmıştır. Test sonucunda tüm hayvanlar negatif bulunmuştur. Sonuç olarak, Border hastalığının epidemiyolojik varlığını ortaya koymak için daha fazla hayvandan daha çeşitli örneklemeler yapılması tavsiye edilmektedir.

Anahtar Kelimeler: Abort, Pestivirus, Border hastalığı, PCR

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Introduction

Border Disease (BD) disease was first reported in 1959 in sheep and goats in the border region between Wales and England and then spread to the whole world (Hughes et al., 1959). Border disease virus (BDV) is from the genus *Pestivirus* of the family *Flaviviridae* (Fauquet et al., 2005). Although BDV affects a wide variety of animals, it mainly infects sheep and goats (OIE, 2017). It is in the same genus as the classical swine fever virus and Bovine Viral Diarrhea Virus (BVDV) 1 and 2 (Oguzoglu et al., 2009). Various studies are showing that pestiviruses have no host specificity. In this context, it can be said that BDV can infect sheep, pigs, goats, cattle as well as deer and giraffes (Paton 1995; Oguzoglu et al., 2010). BDV is divided into 2 biotypes according to whether it has a cytopathogenic effect (CP) or not (Tabash et al., 2009). In recent studies based on sequence analysis and phylogenetic tree, it has been divided into at least 8 genotypes (Caruso et al., 2017; Cerutti et al., 2019). BDV can be transmitted both horizontally and vertically. Vertical transmission is particularly significant in terms of the epidemiological role of the disease. Infection of the foetus in the early period of pregnancy leads to the birth of "persistently infected lambs". Persistently infected animals infect susceptible animals in the herd with the virus shedding with their secretions and extracts (Dahhir et al., 2019). It is also called "hairy shaker disease" or "fuzzy lamb syndrome" due to abnormal hair formation observed in new-born animals (Oğuzoğlu, 2012). Low birth weight, ataxia, occasional enteric dysfunction, mucosal disease-like lesions have also been reported in BDV-infected sheep and lambs (Berriatua et al., 2006). There are no significant clinical findings observed in adult sheep and goats other than abortion (Thabti et al., 2005). Serological studies conducted have revealed that the seroprevalence of BDV varies between 5% and 50% from country to country or from region to region (Cabezón et al., 2010), (Kaiser et al., 2016), (Saeed 2020). The prevalence of BDV has been reported in many countries such as Turkey, Austria, Japan, and Italy (Ataseven et al., 2006; Krametter-Froetscher et al., 2008; Giangaspero et al. 2011; Giammarioli et al. 2015). In Turkey, it was detected in many regions such as Afyonkarahisar (Gür, 2009), Black Sea region (Albayrak et al., 2012), Kars (Yilmaz et al., 2014), Konya (Avcı, 2010), Eastern and Southeastern regions (Ataseven et al., 2006). Diagnostic methods of BDV include techniques such as virus isolation, immunohistochemistry, ELISA, etc. (Dagleish et al., 2010; Strong et al., 2010; Al-Rubayie and Hasso, 2014). It has been reported that detection of BDV from clinical samples is difficult, but RT-PCR is a very successful molecular technique. It has been stated that RT-PCR enables the detection of pestivirus DNA from various samples such as blood, tissue, serum, and swap (Edmondson et al., 2007).

In this study, the aim was to investigate the presence of BDV by PCR test in blood samples from sheep with abortion problems who were taken to Selcuk University Faculty of Veterinary Medicine Internal Medicine Clinic.

Material and Methods

Leukocyte samples were collected from 75 sheep with abortion problems between the ages of 1 and 5-years who were taken to Selcuk University Faculty of Veterinary Medicine Internal Medicine Clinic between November 2019 and December 2020. Blood samples were taken from the jugular vein and transferred to sterile tubes containing EDTA.

Viral RNA extraction was performed according to the procedure specified by the manufacturer (QIAamp Viral RNA Mini Kit/ Cat No./ID: 52906) in the kit. The primer used was the RNA fragment (288bp) of the 5' NC region in the NADL strain of BVDV type 1 reported by Vilček et al. (1994). Primer 324 was 5'ATG CCC WTA GTA GGA CTA GCA 3' (position at NADL 108-128, W= A or T) and primer 326 was 5' TCA ACT CCA TGT GCC ATG TAC 3'(NADL 395-375). QIAGEN OneStep RT-PCR Kit (Cat No./ID: 210212) was used for PCR. Positive control, negative control and 50µL final reaction mixture for each sample (10µl 5x, 1 µl dNTP (10mM), 2 µl forward primer (10pmol), 2 µl reverse primer (10pmol), 1 µl enzyme mix, 29 µl water, 5 µl RNA) were prepared. The thermal cycle program is 1-minute denaturation at 94 ˚C, 1-minute annealing at 56 ˚C, 1-minute extension at 72 ˚C. After 35 cycles performed in this manner, the PCR products were run on a 2% agarose gel. PCR bands were examined under UV light in the presence of ethidium bromide.

Ethical approval: All procedures and animal care complied with the guidelines of the Selcuk University Veterinary Faculty Ethics Committee (Ethical approval number 2019/06 on 2019155).

Results

In this study, leukocyte samples taken from sheep with abortion problems were found to be negative in terms of pestivirus antigen.

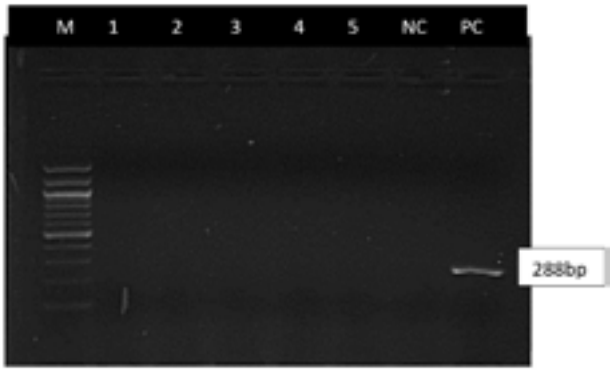

Figure 1. M: Marker, 1: Sample No 1, 2: Sample No 2, 3: Sample No 3, 4: Sample No 4, 5: Sample No 5, NC: Negative Control, PC: Positive Control

Discussion

Pestiviruses are among the most important causes of reproductive disorders and immunosuppression and are responsible for significant economic losses in farms. Bovine Viral Diarrhea Virus (BVDV) and BDV are closely related genetically. Both can infect cattle, sheep, goats, pigs, and non-domestic ruminants (Nettleton and Entrican, 1995). Currently there is no vaccination, control, or eradication program for Border Disease in Turkey.

Abortion observed in sheep may be caused by other infectious agents other than BDV, such as *Neospora caninum* (Dubey and Lindsay 1990), Listeria (Chand and Sadana, 1999), *Brucella* sp. (Ocholi et al., 2005).

Hasırcıoğlu et al. (2009) serologically and virologically investigated the presence of pestivirus in sheep and goats that had abortions. Therefore, they examined serum and leukocyte samples from 735 sheep, 35 goats, and tissue samples from 48 abortions/stillborn lambs by Enzyme-Linked ImmunoSorbent Assay (ELISA). As a result, 475 (64.6%) of 735 sheep with abortion and 2 (5.7%) of 35 goats with abortion were found to be positive for pestivirus antibodies. Besides, they stated

Table 1. The ages of the animals sampled

Number	Age of Animal	Number	Age of Animal
1	3 year-old	39	1.5 year-old
2	5 year-old	40	1.5 year-old
3	5 year-old	41	2 year-old
4	1 year-old	42	2 year-old
5	1 year-old	43	1.5 year-old
6	3 year-old	44	2 year-old
7	2 year-old	45	3.5 year-old
8	1 year-old	46	4 year-old
9	4 year-old	47	4.5 year-old
10	5 year-old	48	3 year-old
11	1.5 year-old	49	2 year-old
12	2.5 year-old	50	2.5 year-old
13	3 year-old	51	4 year-old
14	3 year-old	52	1.5 year-old
15	1.5 year-old	53	3 year-old
16	5 year-old	54	1 year-old
17	4.5 year-old	55	2.5 year-old
18	4 year-old	56	2 year-old
19	3 year-old	57	4 year-old
20	5 year-old	58	3 year-old
21	5 year-old	59	2 year-old
22	1.5 year-old	60	2.5 year-old
23	1.5 year-old	61	3 year-old
24	1 year-old	62	3 year-old
25	2.5 year-old	63	1.5 year-old
26	3 year-old	64	5 year-old
27	4 year-old	65	1 year-old
28	2 year-old	66	4 year-old
29	4.5 year-old	67	1.5 year-old
30	3 year-old	68	1 year-old
31	2 year-old	69	2 year-old
32	4 year-old	70	1 year-old
33	1.5 year-old	71	3 year-old
34	2 year-old	72	1 year-old
35	3 year-old	73	5 year-old
36	2 year-old	74	4 year-old
37	1 year-old	75	2.5 year-old
38	3 year-old		

that while the presence of pestivirus antigen was detected in 5 (0.7%) of the leukocyte samples collected from sheep with abortion and in the waste foetus tissue samples of these animals, they could not detect the presence of antigen in any of the goats with abortion.

Berber and Sözdutmaz (2013) investigated the presence of pestivirus by ELISA in abort samples obtained from sheep in Elazığ, Malatya, and Tunceli provinces. They determined 36 (22.5%) of 160 samples as positive.

Ural and Erol (2017) serologically and virologically investigated pestivirus infection in sheep and goats in Aydın and İzmir provinces. They found that 41.40% (53/128) of goat samples and 47.59% (158/332) of sheep samples were seropositive, but whole blood samples were negative in terms of antigen. They pointed out that although no viraemic animals were detected in sheep and goats, the detection of seropositivity indicated that pestiviruses were circulating in the region.

To investigate the presence of persistent pestivirus in sheep and lambs infected with Contagious ecthyma (CE) infection in a sheep farm in Sakarya Gülyaz (2016) examined leukocyte samples from 18 sheep and 26 lambs by nested RT-PCR method and found negative for the presence of pestivirus nucleic acid.

Avcı (2010) found 11 out of 1000 sheep leukocyte samples (1.1%) as positive for the presence of BDV antigen, while 989 (98.9%) were negative. All 500 lamb leukocyte samples were determined as negative for the presence of BDV antigen.

In the study by Valdazo-González et al. (2006), they sampled 423 sheep, including 5 herds, and detected only 11 (2.6%) as virus-positive. In the second sampling they performed to confirm the presence of persistent infection, 6 of them (1.4%) were found positive. Braun et al. (2014) tested 1170 sheep for BDV by RT-PCR in their study to investigate whether BDV was transmitted to cattle from sheep grazing on the same pasture, and they found that only 8 (0.68%) were positive. Mokhtari ve Manshoori (2018) found that 9 (9%) of the foetal fluids from 100 sheep that had abortions were positive. Braun et al. (2013) found 2,384 sheep negative for BDV by quantitative RT-PCR, while 310 (13.5%) of 2291 sheep were found to be positive for BDV antibodies by ELISA. The antigen results of this study are similar to ours. However, we could not comment on the disease history because we did not examine it serologically. Kaleibar et al. (2014) found 10 (11.36%) out of 88 sheep positive by nested RT-PCR. The reason for this might be the increase in virus titer as they inoculated leukocyte samples into cell culture in their study. Ali et al. (2015) examined a total of 382 pneumonic lung tissues, including 305 sheep and 77 goats, for pestivirus antigen by ELISA, they found 32 (10.5%) sheep and 9 (11.7%) goats positive.

In Burgu's (2003) study, viremia due to pestivirus was found in 1 out of 108 fetuses. It was found that as a result of pestivirus control of other organs of this fetus, the presence of pestivirus was detected in the lung, liver, brain, intestine, and kidney of the same fetus as a result of control of other organs of that foetus. Pestivirus was detected from the amniotic fluid and placentoma samples of the mother of the same fetus, the leukocyte sample was found to be negative.

Burgu et al. (2001) examined a total of 1460 sheep in 9 different farms for persistent infections, they found that only 1 sheep was "transiently viraemic" and none was persistently infected. Therefore, they interpreted that although there were no acute or persistent infected animals in the herd at the time of sampling, there may have been acutely infected animals in the herd before.

Lambs are protected from disease until about 2 months of age by the passive immunity provided by high-quality colostrum they will receive immediately after birth. However, in order to have IgG in the colostrum the animals should be vaccinated before birth in relation to this factor. To prevent BDV, persistently infected animals should be identified and removed from the herd while healthy animals should be vaccinated. Double sampling should be done to identify animals with PI. This should include re-sampling animals that are positive for BDV antigen 1 month after the initial analysis. Those whose tests are positive again should be considered persistently infected and sent to the slaughterhouse. Vaccination aims to clinically protect seronegative animals and to prevent or at least limit the birth of PI lambs, along with protecting

foetuses in the early stages of pregnancy. Depending on the individual serological status of the animals, vaccination should be administered to the entire herd or only to seronegative animals. Sudden temperature changes, transport, weaning, transfer, sudden change in care and feeding conditions are the main stress factors for the animals. Since the expected level of immunity from the vaccine may not be achieved in animals exposed to stress, it is recommended that vaccinations be given during the period when stress in the herd is lowest. Currently there is no commercially available vaccine for BDV. The vaccines against BVDV in Turkey are administered to sheep at half or a quarter of the dose used for vaccination of cattle.

It should be ensured that illegal animal transport, which plays a major role in the spread of BDV, is also brought under control.

In summary, the study evaluated the leukocyte samples from 75 animals and it is recommended that the negative results obtained should be evaluated in parallel with the study conducted by Burgu (2003) along with leukocyte samples in amniotic fluid and placenta samples. It is recommended that a large number of samples from a wide population be tested to investigate the presence of BDV. Eradication programs should be developed to identify and remove persistently infected animals from the flock. It should be noted that sheep may serve as reservoirs for pestiviruses observed in other species. In addition, the activities of the animals (through introduction of illegal animals, transfers between them or imports from neighbouring countries) should be carefully monitored. This will prevent the spread of the disease to larger populations. In addition, animal breeders and veterinarians should be informed about this infection and thus cases should be screened for BDV. The informative study results will contribute to the economic benefits of the country as well as scientific data. In studies conducted on molecular genetic typing of BDV in our region, this will be an important step for studies on vaccination which is the most effective method to prevent the disease.

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Research Article

Prevalence and Antibiotic Resistance of Salmonella sp., E. coli O157, and L. monocytogenes in Meat and Dairy Products

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ABSTRACT

Bacteria-related food poisoning is the most prevalent cause of food borne diseases, reaching up to 33 percent of the incidents. The study aimed to provide data for the risk assessment processes focusing on poisoning due to the consumption of foods of animal origin. Bovine meat, chicken meat, white cheese, and kosher samples were collected from five different stores in Tekirdağ, Turkey, monthly for twelve consecutive months, from January 1 to December 31, 2019. Ten samples of bovine meat, ten samples of chicken meat, ten samples of white cheese, ten samples of kosher cheese, weighing 300 grams each, were collected at each monthly visit. The total number of samples collected for the study was 480. Results showed that 4,16% of the samples (n=12) were contaminated with *Salmonella* sp. (n=12), *Escherichia coli* O157 (n=4), and *Listeria monocytogenes* (n=6). Antibiotic sensitivity studies revealed that the microorganisms were resistant to 8 different antibiotics. Despite the low number of pathogens isolated, their presences with the high antibiotic resistance rates pose a significant threat to public health.
Keywords: Antibiotic resistance, E. coli O157, L. monocytogenes, Salmonella sp.

Et ve Süt Ürünlerinde Salmonella sp., E. coli O157 ve L. monocytogenes'in Prevalansı ve Antibiyotik Direnci

ÖZET

Dünya çapında önemli sorunlardan biri gıda zehirlenmeleridir. Çalışmalar gıda zehirlenmelerinin yaklaşık %33'ünün bakterilerden kaynaklandığını göstermiştir. AB Fasıl 12 kapsamındaki risk değerlendirme konusunda kaynaklık edebilecek veri hazırlamak amacıyla Tekirdağ ilindeki hayvansal ürünlerden *Salmonella* sp., *E. coli* O157, *L. monocytogenes* izolasyonu ve suşların antibiyotik duyarlılıklarının tespiti yapılmıştır. Bu amaçla materyal olarak 2019 yılı içerisinde her ay 10'ar adet ve her biri 300'er gram sığır eti, tavuk eti, beyaz peynir ve kaşar peyniri örnekleri Tekirdağ ilindeki marketlerden alınmıştır. Çalışmada toplam 480 numune üzerinde çalışılmıştır.Yapılan analizler neticesinde 12 adet *Salmonella* sp., 4 adet *E. coli* O157, 6 adet *L. monocytogenes* izole edilmiştir. Çalışmanın ikinci aşamasında izole edilen bakterilerin 8 farklı antibiyotiğe direnç gösterdiği tespit edilmiştir.Çalışma sonucunda Tekirdağ ili için izole edilen bakterilerin sayısal olarak düşük olması sevindirici olarak değerlendirilse de, yüksek antibiyotik dirençliliği halk sağlığını tehdit etmesi bakımından önemli bir sonuç olarak karşımıza çıkmaktadır.
Anahtar Kelimeler: Antibiyotik dirençliliği, E. coli O157, L. monocytogenes, Salmonella sp.

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Introduction

Food poisoning is a significant problem in all countries. The general definition is the ill condition developed by consuming food or water contaminated with pathogenic microorganisms. The reports show that bacteria-related food poisoning is the most prevalent cause of foodborne illnesses, reaching up to 33 percent of the incidents (Çakıcı et al., 2015).

The increase in the food variety and distribution accelerates food poisoning incidence . The *Salmonella* sp., *Escherichia coli* O157, and *Listeria monocytogenes* are the leading causes of food poisoning (Dorman et al., 2010).

Salmonella is a genus of rod-shaped Gram-negative bacteria belonging to the family *Enterobacteriaceae. Salmonella* species are intracellular pathogens and can invade different cell types, including epithelial cells, M cells, macrophages, and dendritic cells (Jantsch et al., 2011). The microorganism has an optimum growth temperature of 37 ºC with a vast range between 7 and 48 ºC and resistance to destruction by freezing (Sorrells et al., 1970). The optimum pH for the growth of *Salmonella* is between 6.5 and 7.5, ranging between 4.5 and 9.0 (Keerthirathne et al., 2016). The species might cause severe infections with severities varying from mild to fatal. Food consumption contaminated with *Salmonella* sp. develops different poisoning conditions, including a mild course or a severe form (Yildirim et al., 2016).

Salmonella species can be found in the digestive tracts of animals. Food or water, previously contacted with the faeces of infected animals, becomes a significant source for transmission of the microorganism if consumed by humans (Goldrick, 2003).

Dairy products and inadequately heat-treated derivatives are the risk-bearing food products for *Salmonella* poisoning (Akkaya & Alişarlı, 2006). The reports indicate that *Salmonella* causes an economic burden of 3.4 billion USD and a billion USD for the USA and Canada, respectively (Yildirim et al., 2016).

Escherichia coli (E. coli) is a Gram-negative, facultative anaerobic, nonspore forming, rod-shaped bacteria belonging to the *Enterobacteriaceae* family. The *E. coli* responsible for gastrointestinal diseases are called DEC, meaning diarrheagenic. Intestinal pathotypes are enterotoxigenic *E.coli* (ETEC), enteropathogenic *E.coli* (EPEC), Vero-toxin or Shiga-toxin producing *E.coli* (VTEC-STEC), enterohemorrhagic *E.coli* (EHEC), enteroaggregative *E.coli* (EAEC), enteroinvasive *E.coli* (EIEC), diffuse adherent *E.coli* (DAEC) (OmerovİC et al., 2017).

Among over a hundred serotypes, *E. coli* O157 of the EHEC group is the most prevalent serotype causing food poisoning. Bovine and sheep are the main reservoirs. *E. coli* O157 causes hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TP) in humans. Besides, due to the resemblance to the toxin of Shigella dysentery type I, the verotoxin produced by the microorganism is called Shiga like toxin (SLT) increases its significance (Temelli, 2002).

The primary source of *E. coli* O157 is the gastrointestinal tract of the bovine and sheep. The organism's isolation rate in the faeces of the bovine and sheep might rise to 10%. *E. coli* O157 infections are caused by consuming water or food contaminated with faeces (Akkaya et al., 2007).

Listeria monocytogenes (*L. monocytogenes*) is a pathogenic, Gram-positive, rod-shaped, motile between 18-26 oC, nonspore forming, non-capsulated, intracellular bacteria (Berktaş et al., 2006). There are 13 serotypes of *L. monocytogenes* able to develop the disease. However, more than 90% of human isolates belong to serotypes 1/2a, 1/2b, and 4b. The serotype 4b strains are responsible for 33 to 35% of sporadic human cases (Ward et al., 2004). The microorganism can be isolated from humans, domestic animals, raw agricultural and fishery products (Lakicevic et al., 2015). The organism can be found in the animal's flesh, blood, and milk, regardless of the listeriosis symptoms. The infection might occur with raw and undercooked food, including cross-contamination following cooking procedures. Vegetables contaminated with sewage or soil fertilized with animal manure are other transmission sources with *L. monocytogenes* (Ekİcİ et al., 2018).

The resistance to antibiotics has become a global public health concern. Besides the acceleration in the resistance to commonly used broad-spectrum antibiotics, the reports show that new drugs have been quickly followed by the emergence of resistant strains (Hoge et al., 1998). In addition to developing resistant pathogenic bacteria, the administration of an antibiotic alters the gut microbiota and creates antibiotic-resistant intestinal bacteria that enable genetic transfer to the pathogenic bacteria and facilitate multi-drug resistance. Seemingly paradoxically, the broad and intensive use of antibiotics creates a bigger drug-resistant world in which the antimicrobials are less useful (Atabey, 2011).

Aiming to provide data for the risk assessment processes on poisoning due to the consumption of foods of animal origin, the study analyzed *Salmonella* sp., *E. coli* O157, and *L. monocytogenes* by isolation of the organisms from the meat and dairy products sold in Tekirdağ, Turkey and the analysis for antibiotic resistance.

Material and Methods

The study included samples collected from five different stores in Tekirdağ, Turkey, monthly for twelve consecutive months, from January 1 to December 31, 2019.

Ten samples of bovine meat, ten samples of chicken meat, ten samples of white cheese, ten samples of kosher cheese, weighing 300 grams each, were collected at each monthly visit. The total number of samples collected for the study was 480.

Bovine, chicken, white cheese and kosher cheese samples were taken in original packages and carried to the laboratory in cold-chain (4 ºC) containers.

Salmonella sp. isolation and identification were performed according to the ISO 6579-1 standard. *E. coli* O157 isolation and identification was performed according to the ISO 16654:2001 standards. *L. monocytogenes* isolation and identification was performed according to the ISO 11290-1 standard.

The isolated pathogens were cross-checked, and verification was confirmed by the VIDAS method. VIDAS verification was performed according to the ISO 16140-1:2016 standards. An automated, multi parametric immunoassay system which uses ELFA (Enzyme-Linked Fluorescent Assay) Phage and Immuno concentration technology, VIDAS® Salmonella (SLM) Immunoassay Method with Rappaport-Vassiliadis (RV) Medium, VIDAS Listeria monocytogenes II (LMO2), and VIDAS® E. coli O157 (ECO) and O157:H7 Plate Method were used for qualitative analysis.

Antibiotic sensitivity test

The antibiotic resistance of the pathogens obtained within the scope of the EUCAST 2018 standard was examined using the disk diffusion technique. Samples yielding positive results were incubated in Mueller-Hinton agar at 37 ºC for 24 hours. The

Table 1. Samples with positive results by months

	Salmonella sp.				*E. coli* O157				*L. monocytogenes*			
	Bovine	**Chicken**	**White cheese**	**Kosher cheese**	**Bovine**	**Chicken**	**White cheese**	**Kosher cheese**	**Bovine**	**Chicken**	**White cheese**	**Kosher cheese**
January		1										
February											1	
March	1											
April	1				1							
May		2							1		1	
June	1				1							
July		1										
August		1			1							
September		2			1						1	1
October												
November		1							1			
December		1										

bacterial inoculum was adjusted with SSF to the Mac Farland Nº 0,5 turbidity standard. Every inoculum was spread over plates containing Mueller-Hinton agar and a paper filter disc (6 mm). The inhibitory zone diameter of the discs were measured in mm scale. The diagnostic discs used in antibiogram tests were Ampicillin (AMP 25 µg), Ciprofloxacin (CIP 10 µg), Nalidixic acid (NA 30 µg), Streptomycin (S 25 µg), Gentamicin (CN 30µg), Sulphafurazole (SF 300 µg), Trimethoprim (W 5µg), Chloramphenicol (C 30µg), Tetracycline (TE 30µg), Colistin (CT 25µg), Cefotaxime (CTX 30µg), Ceftazidime (CAZ 30µg), Cefoxitin (FOX 30µg), Cefepime (FEP 30µg), Meropenem (MEM 10µg).

Results

Among the 480 samples, 22 (4.6%) was contaminated with the study microorganisms. *Salmonella* sp. was found in 12 (2.5%), *E. coli* O 157 was found in 4 (0.83%), and *L. monocytogenes* was found in 6 (1.25%) of the samples. In Table 1, the samples with positive results by months were presented.

Salmonella sp. was isolated from 3 (2.5%) bovine samples and 9 (7.5%) chicken samples. There were no white cheese and kosher cheese samples with positive results for *Salmonella* sp. The identification of *Salmonella* species isolated from the bovine samples showed that two samples included *S.* Typhimurium, and one specimen had *S. Bongori*. In chicken samples, *S.* Enteriditis and *S.* Typhimurium were identified in four and three samples, respectively. *S. Infantis* and *S. Dublin* were identified in one specimen for each.

E. coli O 157 was found in four (3.33%) of the bovine specimen and was not isolated from the chicken meat, white and kosher cheese samples.

L. monocytogenes was isolated from two (1.66%) bovine, three (2.5%) white cheese and one (0.83%) kosher cheese samples. There were no chicken samples positive for *L. monocytogenes*.

The isolated pathogens were cross-checked, and 100% verification was confirmed by the VIDAS method.

The antibiotic resistance study showed that the *S. Enteritidis* and *L. monocytogenes* strains had the maximum variety of antibiotic resistance with 11 different antimicrobials. *S. Typhimurium* was found resistant to 8 different antibiotics. *S. Infantis* and *S. Dublin* strains showed resistance to 5 different antibiotics each. *E. coli* O157 strains showed resistance to 10 and *L. monocytogenes* to 11 different antimicrobials. From the antibiotic resistance point of view, Streptomycin was the only antimicrobial to which all strains were found resistant. *S. Enteritidis* was the only microorganism that presented antibiotic resistance to gentamycin, ceftazidime and cefepime. Presenting the least number of antimicrobial resistance, *S. Dublin* was the only microorganism resistant to cefotaxime. *E. coli* O157 strains presented resistanceto colistin, and *L. monocytogenes* was the only isolate detected to be resistant to chloramphenicol. In Table 2, the samples with strains showing antibiotic resistance were presented.

Discussion

In our study, *Salmonella* sp. were isolated from 3 (2.5%) bovine samples. The reports conducted in Turkey show that the *Salmonella* sp. isolation rate found in the study were similar to the rates in others ranging between 2.5 and 6%. Büyükünal et al., (2015), Kahraman T & Aydın, (2009), and Yildirim et al., (2016) included an approximately similar number of samples and isolated *Salmonella* spp. from the bovine samples with rates of 1%, 2.5% and 6%. The studies from other countries presented similar rates with bovine specimen numbers ranging from 200 to 72292 samples, between 1.5% to 5% (Madden et al., 2001),(Zarei et al., 2013). It was worth noting that the isolation rates inclined as the sample size increased in the researches.

Salmonella sp. were isolated from 9 (7.5%) chicken samples. Studies conducted in Turkey with approximate sample sizes indicate comparable results. Tanoğlu & Gümüşsoy, (2008), Kahraman & Aydın, (2009) and Acaröz et al., (2018) reported similar isolation with 9.7%, %3, and %2.9, respectively. The researches from other countries with similar sample sizes present parallel to our results. One high rate was reported from South Africa, indicating that 19% of the samples were contaminated with *Salmonella* (van Nierop et al., 2005).

In white and kosher cheese samples, *Salmonella* sp. was not detected. There were studies conducted in Turkey reporting the same results Turantaş et al., (1989), Gülmez & Güven, (2001). However, Kahraman et al., (2010) and Akkaya & Alişarlı, (2006), have presented similar rates of *Salmonella* presence in cheese samples with 1.9 and 2%.

In our study, *E. coli* O157 was detected only in 4 (3.33%) bovine samples. The researches of Bingöl et al., (2013), Fantelli, (2001) and Büyükünal et al., (2015) reported that no *E. coli* O157 was found in bovine samples. However, Ahmed & Shimamoto,

Table 2. The number and percentages of samples with strains showing antibiotic resistance

	S. Enteritidis		*S.* Typhimurium		*S.* Bongori		*S.* Infantis		*S.*Dublin		*Total Salmonella* sp.		*E. coli* O157		*L. monocytogenes*	
	n	*4*	*n*	*5*	*n*	*1*	*n*	*1*	*n*	*1*	*n*	*12*	*n*	*4*	*n*	*6*
		%		*%*		*%*		*%*		*%*		*%*		*%*		*%*
Ampicillin	4	100,00%	3	60,00%	1	100,00%	0	0,00%	1	100,00%	9	75,00%	4	100,00%	6	100,00%
Ciprofloxacin	1	25,00%	1	20,00%	0	0,00%	1	100,00%	1	100,00%	3	25,00%	3	75,00%	6	100,00%
Nalidixic Acid	2	50,00%	1	20,00%	0	0,00%	0	0,00%	1	100,00%	4	33,33%	2	50,00%	6	100,00%
Streptomycin	3	75,00%	2	40,00%	1	100,00%	1	100,00%	1	100,00%	7	58,33%	3	75,00%	6	100,00%
Gentamycin	2	50,00%	0	0,00%	0	0,00%	0	0,00%	0	0,00%	2	16,67%	0	0,00%	0	0,00%
Sulphamethoxazole	4	100,00%	3	60,00%	1	100,00%	1	100,00%	0	0,00%	8	66,67%	3	75,00%	5	83,33%
Trimethoprim	4	100,00%	3	60,00%	1	100,00%	1	100,00%	0	0,00%	8	66,67%	4	100,00%	4	66,67%
Chloramphenicol	0	0,00%	0	0,00%	0	0,00%	0	0,00%	0	0,00%	0	0,00%	0	0,00%	4	66,67%
Tetracycline	0	0,00%	0	0,00%	1	100,00%	0	0,00%	0	0,00%	1	8,33%	3	75,00%	6	100,00%
Colistin	0	0,00%	0	0,00%	0	0,00%	0	0,00%	0	0,00%	0	0,00%	1	25,00%	1	16,67%
Cefotaxime	0	0,00%	0	0,00%	0	0,00%	0	0,00%	1	100,00%	1	8,33%	0	0,00%	0	0,00%
Ceftazidime	1	25,00%	0	0,00%	0	0,00%	0	0,00%	0	0,00%	1	8,33%	0	0,00%	0	0,00%
Cefoxitin	2	50,00%	1	20,00%	0	0,00%	1	100,00%	0	0,00%	3	25,00%	1	25,00%	6	100,00%
Cefepime	1	25,00%	0	0,00%	0	0,00%	0	0,00%	0	0,00%	1	8,33%	0	0,00%	0	0,00%
Meropenem	2	50,00%	1	20,00%	0	0,00%	0	0,00%	0	0,00%	3	25,00%	4	100,00%	2	33,33%

(2014) and Zarei et al., (2013) have reported similar isolation rates to our study with 2.6 and 2.8%, respectively.

There were no chicken and cheese samples contaminated with *E. coli* O157 among the collected samples. In a similar study, Manguiat & Fang (2013) and Jo et al., (2004) have reported that cheese samples were uncontaminated with *E. coli* O157. Also, Arslan & Özdemir, (2008) and Gonzalez et al., (2000) reported the same negative contamination results for chicken samples.

There were only two (1.66%) bovine samples contaminated with *L. monocytogenes*. Similar results have been reported from studies conducted in Turkey and other countries Büyükünal et al., (2015), Kahraman T & Aydın, (2009), Iannetti et al., (2016). A study conducted by Vitas & Garcia-Jalon, (2004) reported that 34.9% of the meat samples were contaminated with *L. monocytogenes*.

The chicken samples in our study were not contaminated with *L. monocytogenes*. Similar results were reported in studies of Yerlikaya (2015), Sağlam (2011). Vitas & Garcia-Jalon, (2004) isolated *L. monocytogenes* from 36.1% of the chicken samples, reporting a very high number similar to the result with bovine samples. However, Mena (2004) have reported that 60% of the chicken samples were contaminated with *L. monocytogenes*, expressing an extreme rate.

Among the cheese samples, *L. monocytogenes* was isolated from three (2.5%) white and one (0.83%) kosher cheese. The researches conducted by Aygun & Pehlivanlar (2006), and Çiftçioğlu & Uğur (1991), analyzing white cheese, indicate comparable findings with 2.85% and 2.9%. In the study of Ekİcİ et al. (2018), 3% and Kahraman et al. (2010) 1.7% of the kosher cheese samples were contaminated with *L. monocytogenes*, similar to our findings.

There might be various reasons for explaining the different isolation rates in the studies. There could be too many probable contamination points to consider, beginning from feeding the animals to the cleaning and disinfection procedures of the packaging and the shelves the products were kept. Also, sampling methods should be considered. Therefore the isolation results might be evaluated regionally or even locally to reach reliable interpretations.

The findings of the antibiotic resistance of the strains showed a variety of results. Streptomycin was the only antimicrobial to which all the microorganisms showed resistance.

A study conducted by Şahan et al. (2016) showed that among *S. Infantis* isolates, resistance to nalidixic acid, sulfonamides, tetracycline, and trimethoprim rates were 92.7%, 92.3%, 88.3%, and 78.6%, respectively. The resistance rates in *S.Typhimurium* isolates for sulfonamides, ampicillin, and trimethoprim were 65%, 47%, and 35%, respectively. The nalidixic acid and sulfonamide resistance in the *S.Enteriditis* strains were 75% and 92%, respectively. The research by Bozkurt (2018) showed that all of the *Salmonella* sp. were resistant to penicillin and 66% to ampicillin and erythromycin. Similar high rates for *Salmonella* sp. were reported in a study by Yang et al. (2020) with 72.3% to nalidixic acid, 55.3% to ampicillin and 48.7% to streptomycin.

The antibiotic resistance analysis of *E. coli* conducted by Dursun (2008) showed that the resistance rates for cefalotin, chloramphenicol, sulphametaxosole-trimethoprim, erythromycin, and amoxicillin-clavulanic acid were 100%, 87.5%, 81.25%, 81.25%, and 62.5%, respectively. Various studies highlighted *E. coli* O157 showing resistance to streptomycin, cefalotin, tetracyclines, amikacin, cefotaxime, erythromycin, chloramphenicol, nalidixic acid, neomycin, ofloxacin (Çadırcı et al., 2017; Elafify et al., 2020).

Alonso-Hernando et al. (2012) have reported an increase in the rate of one or more antibiotic-resistant *L. monocytogenes* isolates from 37.2 % to 96% in 13 years. Interestingly, in both studies conducted in 1993 and 2006, the nalidixic acid resistance rate was 100%. In the study, the multi-drug resistance among *L.*

monocytogenes isolates was reported to increase from 18.6% to 84% in the time frame. Regarding resistance to enrofloxacin and ciprofloxacin, the rates were increased from 23.3% and 25.6% to 68% and 52%. Alonso-Hernando et al. (2012) indicate that in addition to the number of antibiotics *L. monocytogenes* isolates were resistant to, the percentage of the strains with resistance also increased. A similar study conducted by Maung et al. (2019) presented a five-year difference in the *L. monocytogenes* strains' resistance to fosfomycin and oxacillin between 2012 and 2017. The resistance to fosfomycin and oxacillin were increased from 57.3% and 72% to 95.7% and 82%, respectively. The multi-drug resistance rate of the *L. monocytogenes* strains rose from 46.7% to 82.62%.

The previous studies show similar results to our findings. In recent years, reports highlight the increase in the strength and variety of antibiotic resistance of the pathogenic bacteria.

The study results for the Tekirdağ region might include low isolate numbers. Nevertheless, the findings indicate a significant risk to public health and should be in consideration. Also, the increased rate and variety in antibiotic resistance results boost the amount at public health risk.

At this point, the careful prescription of antibiotics to animals with performing antibiogram tests, avoiding misuse and repetition of the same molecule, and following gold-standard treatment methods might decelerate antibiotic resistance rates. Moreover, following HACCP processes in the production of aminal foods such as adequate sanitation conditions and sufficient thermal procedures might help eliminate pathogenic bacteria at the source.

Conflict of Interest

The authors declare no conflicts of interest.

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Research Article

Ultrasonographic Measurements of the Bulbus Oculi of the Camel

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ABSTRACT

Ophthalmic ultrasonography is considered a useful modern tool to quantify the ocular dimensions. The main aim of this study was to give information about the ultrasonographic measurements of the normal hybrid camel eye. Besides, to calculate some indices of the camel eye and discuss them in comparison with the ocular measurements of other animals reported previously. Fourteen formalin-preserved eyeballs were subjected to corneal ocular ultrasonographic examination in horizontal imaging plane. The ultrasonographic results of the eyeballs showed 95% confidence intervals for measurements as corneal thickness (CT) (1.56-1.87), anterior chamber depth (ACD) (2.33-4.27), lens thickness (LT) (6.81-10.00), vitreous chamber depth (VCD) (23.01-24.44), axial length (AL) (35.13-38.60), and optical axis (OA) (34.89-37.24). Indices also showed that 95% confidence interval ranges were as CT/AL (0.04-0.05), ACD/AL (0.06-0.11), LT/AL (0.19-0.26), VCD/AL (0.62-0.66) and OA/AL (0.96-1.00). This knowledge of the normal ocular dimensions may especially be helpful in the diagnosis of the deviation from normal eye and progression towards any ocular problem in the camel.

Keywords: camel, corneal index, eyeball, ophthalmology, ultrasound

Devede Bulbus Oculi'nin Ultrasonografik Ölçümleri

ÖZET

Oftalmik ultrasonografi, oküler boyutları ölçmek için kullanışlı modern bir cihaz olarak kabul edilmektedir. Bu çalışmanın ana amacı, melez deve gözünün ultrasonografik ölçümleri hakkında bilgi vermektir. Bunun yanında deve gözünün bazı indekslerini hesaplamak ve bunları daha önce kayıt altına alınmış hayvanların oküler ölçümleriyle karşılaştırarak tartışmaktır. Formalin ile fikzasyonu yapılmış 14 göz küresi, horizontal görüntüleme düzleminde korneal oküler ultrasonografik incelemeye tabi tutuldu.

Göz kürelerinin ultrasonografik sonuçları (%95 güven aralığında); kornea kalınlığı (CT) (1.56-1.87), ön kamara derinliği (ACD) (2.33-4.27), lens kalınlığı (LT) (6.81 10.00), vitröz kamara derinliği (VCD) (23.01-24.44), eksenel uzunluk (AL) (35.13-38.60) ve optik eksen (OA) (34.89-37.24) olarak bulundu.

Ayrıca indeksler (%95 güven aralığında); CT/AL (0,04 0,05), ACD/AL (0,06-0,11), LT/AL (0,19-0,26), VCD/AL (0,62-0,66) ve OA/AL (0.96-1.00) hesaplandı. Devede, oküler boyutların bilinmesi, normal göz boyutlarından sapmalar ve herhangi bir oküler sorunun ilerlemesinin teşhisinde yardımcı olabilir.

Anahtar kelimeler: Deve, korneal indeks, göz küresi, oftalmoloji, ultrason

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Introduction

The knowledge of ocular status and visual acuity is important factor for the good performance of any animal. Optical biometry is an important technique for the assessment of normal healthy eyes and appendages but it has a limitation of requiring good optical pathway to get accurate results. It seems impossible to meet this criteria in some pathological conditions. The ophthalmic ultrasonography is considered useful modern tool to quantify the ocular dimensions instead of direct clinical measurements. This technique is preferred because of its safety and non-invasiveness (Dudea, 2011). So, ultrasonography seems a good approach where use of ophthalmoscopy is unimpressive (Holladay, 2009).

A-mode (amplitude modification) is most commonly used in research oriented studies (Oliver, 2008) but B-mode (brightness modification) ultrasonography is preferred in clinical ophthalmology for two-dimensional cross-section unveiling of an organ. It shows amplitude of returned echoes as dots (Mirshahi et al., 2014) while A-mode shows amplitude as spikes on horizontal line (Oliver, 2008). A probe of the B-scan can also generate charismatic image of the eye as it transmits multiple sound waves (Solarte & Shaikh, 2007). Another crucial component of these devices is transducer and generally, linear transducers having 7.5 to 10 MHz frequency are used for ocular measurements (Dudea, 2011).

Improvements in the technology has proved ocular ultrasound biometry as useful tool in different animals like dolphin (Cartee et al., 1995), goat (Ribeiro et al., 2009), horse (Gialletti et al., 2018; Sorouri et al., 2009), Indian camel (Kelawala et al., 2015) and also in birds like parrot (Lehmkuhl et al., 2010). There are some studies related to ultrasonography of one-humped camel eyes, even showing comparison with other animals like buffalo (Kassab, 2012). So, the objective of this study was firstly, to get ocular measurements of hybrid camel "*Tülü*" (Çalişkan, 2016). by using ultrasonographic imaging modality and then, figure out some indices and compare those results with already available data so far**.**

Material and Methods

Total 14 eyes of the seven hybrid male camels were obtained from local slaughterhouse. The carcass weight of the camels were 380.29 ± 45.45 kg. The both right and left eyes (n = 14) were collected initially dissecting through the conjunctiva of the upper and lower eyelids. Optic nerves were detached close to their exit from eyeballs. Newly unfolded camel eyes were then trimmed carefully to remove fat, extra ocular muscles and other tissues. After dissection the eyes were immersed in 10% formaldehyde solution and kept at 4 °C up to the study. Before examination, eyes were kept under running water to remove excess formalin. The eyeballs were then engrossed in water bath and ultrasonography was performed. B-mode ultrasound scanner (Esaote MyLab 30 Vet) was used, which accepted linear transducer with 8 MHz.

Trans-corneal method was applied with gel on surface of cornea to measure different dimensions of eye. The probe was settled at right angle to mid of cornea. The sound waves reflected from eyeball were recorded on attached computer screen. Recordings were made when posterior wall of eyeball was visualized (Kassab, 2012). The corneal thickness (CT) was measured from anterior to posterior surfaces of the cornea. The lens thickness (LT) was calculated from anterior to posterior surface of the lens. The anterior chamber depth (ACD) was assessed from the distance between posterior corneal surface and anterior lens surface. The vitreous chamber depth (VCD) was computed as distance between posterior lens surface and the retina. The axial length (AL) was the straight distance from anterior surface of the cornea to the retina (Kassab, 2012; Osuobeni & Hamidzada, 1999). Optical axis (OA) was measured as the length starting from anterior surface of the cornea up to the optic nerve papilla (Lehmkuhl et al., 2010). All the measurements were made in millimeters. The ultrasonographic image and measurements are shown in the Figure 1 (A and B).

Table 1. Direct measurements (Mean ± Standard Deviation) of the ocular dimensions of the hybrid camel through ultrasonography

Eyeball Measurements	Mean ± SD (mm)	95% Confidence Intervals
CT	1.71 ± 0.66	1.56-1.87
ACD	3.3 ± 1.051	2.33-4.27
LT	8.41 ± 1.73	6.81-10.34
VCD	23.72 ± 0.77	23.01-24.44
AL	36.86 ± 1.87	35.13-38.60
OA	36.06 ± 1.27	24.89-37.24

Corneal thickness **(CT)**, Anterior Chamber Depth **(ACD),** Lens Tickness **(LT),** Vitreus Chamber Depth **(VCD),** Axial Length **(AL),** Optical Axis **(OA).**

Some indices like CT/AL, ACD/AL, LT/AL, VCD/AL and OA/AL were also determined. The statistical analyses was performed using the Statistical Package for the Social Sciences (SPSS) 16.0 for Windows (SPSS Inc, Chicago, IL. USA). The right and left side data were pooled to calculations. Data was expressed as mean ± standard deviation. The 95% confidence intervals were also calculated for all the optical measurements.

Results

Ultrasonographic measurements of right and left eyeballs showed that 95% confidence intervals for CT, ACD, LT, VCD, AL and OA were 1.56-1.87, 2.33-4.27, 6.81-10.00, 23.01-24.44, 35.13-38.60 and 34.89-37.24, respectively. Indices also showed that 95% confidence interval ranges were as 0.04-0.05, 0.06-0.11, 0.19-0.26, 0.62-0.66 and 0.96-1.00, respectively. These values are mentioned along with their means and standard deviations in the Table 1 and 2.

Discussion

Ultrasonographic eye measurements are generally presented as direct dimensions (Kelawala et al., 2015; Osuobeni & Hamidzada, 1999; Yadegari et al., 2013). However, in morphometric evaluations, indices are more reliable than diameters because they are independent of the size (Kara et al., 2011). For this reason, index values for hybrid camel eye ultrasonographic measurements are presented in this study. These results were especially important in the veterinary clinics

Table 2. Indices of the hybrid camel eye calculated from the ocular measurements

Indices	Mean ± SD	95% Confidence Intervals
CT/AL	0.05 ± 0.004	0.04-0.05
ACD/AL	0.09 ± 0.031	0.06-0.12
LT/AL	0.23 ± 0.036	0.19-0.26
VCD/AL	0.64 ± 0.022	0.62-0.66
OA/AL	0.97 ± 0.026	0.96-1.00

for camels, because they deal with different camel breeds of various sizes.

The ultrasonographic appearance of the hybrid camel eye was just like the camel studies reported previously by Kelawala et al. (2015) and Yadegari et al. (2013). The anterior and posterior surfaces of the cornea and the lens appeared to be hyper echoic. The aqueous, vitreous humors and the central part of the lens were seemed anechoic.

As the assessment was made on the indices, corneal thickness index and lens thickness index were measured exactly similar to that of the adult one-humped camel but young camel was having little thinner cornea (Kassab, 2012) as compared to our camel. For camel in their studies, Yadegari et al. (2013) and Kelawala et al. (2015) found very much greater lens index than the recent study. The value of ACD/AL was very much bigger while VCD/AL of adult one-humped camel were found very much smaller in size by Kassab (2012), Kelawala et al. (2015) and Yadegari et al. (2013) than that of the hybrid camel. But the ACD/AL of eye of the hybrid camel appeared same as that of the young camel.

In comparison with other large animals, the calculations indicated that adult buffalo (Kassab, 2012) and Jersey cattle were having same corneal index but Holstein Friesian cattle was having higher than that of the hybrid camel (Potter et al., 2008). In recent study, the measurement of the ACD/AL of the camel was little smaller than the buffalo and goat but similar to the cattle and miniature horse. Unlikely, the VCD/AL was found very much larger in our studied camel than that of the bovine, equine, caprine and Persian cat (Kassab, 2012; Mirshahi et al., 2014; Potter et al., 2008). The Persian cat was having very much smaller ACD/AL than the camel (Mirshahi et al., 2014). The LT/AL of both young and adult buffalo was similar but cattle, horse, goat and cat were having very much bigger LT/AL than the hybrid camel (Kassab, 2012; Mirshahi et al., 2014; Potter et al., 2008; Sorouri et al., 2009).

While looking for the comparison with birds, the parrot showed higher values for both the ACD/AL and LT/AL but very much smaller optical axis index (OA/AL) than the camel (Lehmkuhl et al., 2010). Besides, humans possessed same ACD/AL for the eye (Quan-hao et al., 2007) as the camel.

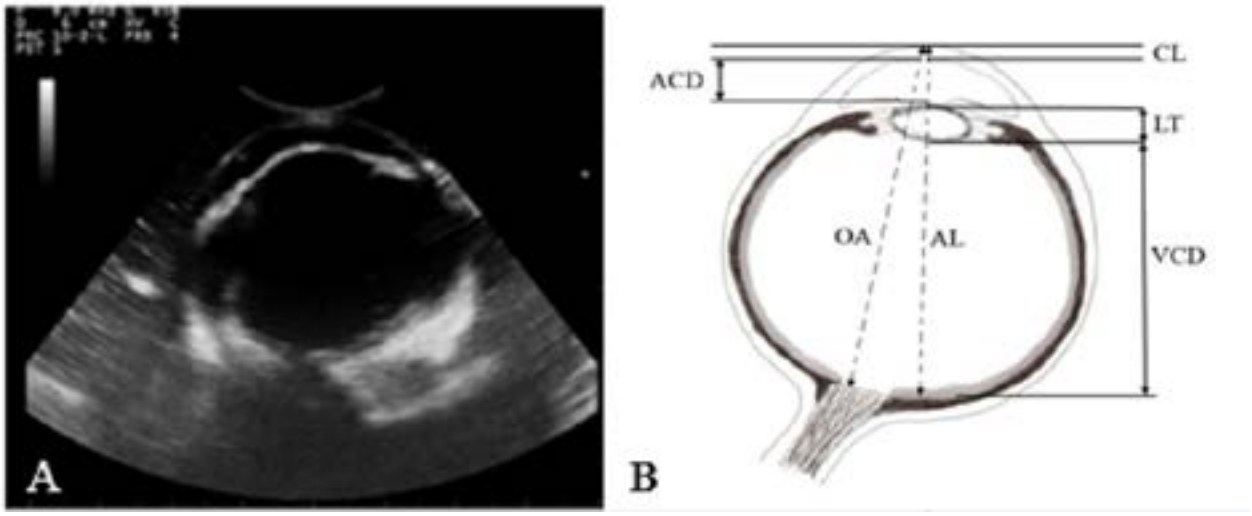

Figure 1 (A). B-mode ultrasonography of the camel showing ocular measurements.

Figure 1 (B). showing schematic diagram of the camel eye measurements. Corneal Thickness **(CT)**, Anterior Chamber Depth **(ACD),** Lens Tickness **(LT),** Vitreus Chamber Depth **(VCD),** Axial Length **(AL),** Optical Axis **(OA).**

In a study by Osuobeni & Hamidzada (1999), available data about the indices showed that the ACD/AL and LT/AL ratios of the hybrid camel were smaller as compared to the humans (0.16), one-humped camel (0.17 and 0.35) horse, cow (0.15 and 0.31-0.34) and cat (0.40). The VCD/AL ratio in present study was perceived greater than the one-humped camel (0.48), horse (0.55) and cow (0.51) but smaller than in the humans i.e. about 0.70.

In general, the animals are having larger ocular dimensions than the human especially thicker lens make them more efficient to focus light on the retina. The differences in measurements between camel and other larger animals could be attributed to the fact that a compensatory mechanism might have been adopted by “the ship of the desert” for the harsh and dry weather conditions, to keep the eyes in proper shape while facing powerful winds over there. The variation between camel

and small animals could be ascribed to the smaller skull and orbit size of them. Moreover, these differences could have been influenced by the age of different animals as seen by Kassab (2012) and Hashemi et al. (2012) in camel and humans, respectively. Besides, there was no impact seen by the gender on eyeball of the goat by Ribeiro et al. (2010), so this concept can be rejected or must be studied in detail for other animals as well.

This study has some limitations that preserved eyeballs had been used and the number of camels was not sufficient. It was seen in the research by Tran et al. (2017), performed recently on the formalin-fixed eye samples, that there was only negligible shrinkage in tissue. Therefore, this fixation point can be neglected. Also, we are giving indices so the shrinkage effect can be ignored.

In conclusion, the present study revealed ocular measurements of the hybrid camel using ultrasound B-mode technique. Ultimately, the knowledge of the optical dimensions will help veterinary clinicians in understanding problems related to the vision of camels. Moreover, ocular ultrasonography could prove to be a complementary technique in diagnosis of the ocular problems in routine clinical cases as well. In future more reliable results could be generated if the limitations of this study could possibly be eradicated. These outcomes should also be compared with the research performed in the live hybrid camel to increase their authenticity.

Acknowledgements

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Conflict of interest

The authors declare that they have no conflict of interest in this study.

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Case Report

Ovarian Metastatic Transmissible Venereal Tumour in a Bitch – A Case Report and Review

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ABSTRACT

Approximately 5% of transmissible venereal tumour can metastatize to various parts of the body. The case presented explains the clinical appearance and histopathology results of the ovarian metastasis of TVT in a female German shepherd. The bitch was brought to the teaching hospital of Ondokuz Mayıs University, Faculty of Veterinary Medicine with a vaginal discharge. A mass with a diameter of 3x1x1 cm was detected on the dorsal vaginal wall. In vaginal cytology, the presence of TVT cells were determined. During ultrasonographic evaluation a mass was detected caudal to the left kidney with irregular margins. The dimension of the mass after ovariohysterectomy was found to be 9x11 cm. Because it was necessary to investigate the possibility of metastasis and to make comparison, the vaginal mass was also removed and sent for histopathological examination and both masses were identified as TVT. According to our knowledge this is the first report presenting a case of TVT with solely ovarian metastasis.

Keywords: bitch; ovarian metastasis; Sticker's sarcoma

Bir Dişi Köpekte Ovaryuma Metastaz Yapan Transmissible Veneral Tümör Olgusu-Olgu Sunumu ve Derleme

ÖZET

Transmissible veneral tümör (TVT) %5 oranında vücudun farklı bölümlerine metastaz yapmaktadır. Sunulan olguda dişi bir Alman çoban köpeğinde TVT'nin ovaryuma metastazı ve klinik görünümü anlatılmaktadır. Uzun süreli kanlı vaginal akıntı şikayetiyle getirilen olgunun vaginal duvarınının dorsalinde 3x1x1 cm çapında kitle belirlendi. Vaginal sitolojide TVT hücreleri görüldü. Ultrasonografik muayenede sol böbreğin kaudalinde, kenarları düzensiz bir kitle tespit edildi. Ovaryohisterektomi sonrası ölçümlerde kitlenin 9x11 cm boyutlarında olduğu belirlendi. Kitlenin metastaz sonucu şekillenme ihtimali nedeniyle, her iki kitlenin karşılaştırılması amacıyla, vaginal kitle de alınarak histopatolojik değerlendirmeye gönderildi. Değerlendirme sonucunda her iki kitlenin de TVT olduğu belirlendi. Yaptığımız araştırma sonucuna göre, sunulan olgu TVT'nin sadece ovaryuma metastaz yaptığı ilk olgu olma özelliğindedir.

Anahtar kelimeler: köpek; ovaryum metastazı; Sticker sarkomu

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Introduction

Tumour cells generally originate from long term genetic and epigenetic changes. First phase is the irreversible genetic differentiation, also called promotion, is followed by the end phase, which is the change of the benign tumour into a malign one and gaining metastatic characteristics. Normally, transmission of tumours with contact is not possible. However transmission of tumours that does not fit that explanation were first discovered in dogs as transmissible venereal tumour (TVT) (in 1820), then Syrian hamsters as contagious reticulum cell sarcoma (in 1961) and Tasmanian devils as devil facial tumour disease (in 1996) (Murchison, 2008). Transmissible venereal tumours have special significance because it was the first to be discovered and it was seen globally. The transference of tumour cells were only discovered at 1995 even though the tumour was believed to be around for 2500 years (Murgia et al., 2006; Temitope et al., 2010). Other tumour types usually occur in later stages of life (6-10 years) though TVT happens at an earlier age (2-5 years) because it happens due to dissimilar mechanisms (Gürel et al., 2002). Transmissible venereal tumour has an incidence of 2.8% among all tumours of the dog (Brodey and Roszel, 1967). While TVT is mostly benign, 5% of the cases have the ability to metastasis. The chance of metastasis in males (16%) is greater than females (2%) (Martins et al, 2005), thereby metastatic TVT cases are especially rare in bitches.

Up until now many canine TVT cases were reported with metastases to many organs and systems like skin and subdermal tissue, mammary glands, brain, eye, palpebral conjunctive tissue, nasal mucosa, soft palate, mediastinum, lungs, liver, spleen, lymph nodes (Higgins, 1966; Adam and Slaughter, 1970; Ayyappan et al., 1994; Baştan et al., 2008; Özyurtlu et al., 2008; Temitope et al., 2010; Behera et al., 2012; Milo and Snead, 2014; Uçmak et al., 2019). To the best of our knowledge the only metastasis of TVT in the reproductive system was the metastasis of TVT to the uterus and ovaries reported by Baştan et al. (2008). This case presented here is the first to report the solely ovarian metastasis of TVT in a bitch. The clinical appearance of the case and the treatment will be explained in this report.

Patient History, Clinical Findings and Treatment

The material of the study was a two years old German shepherd dog weighing 24 kg that was brought to our clinic with a vaginal discharge. The patient's condition got progressively worse, with loss of appetite and lethargy. The bitch had a healthy delivery and litter in the previous cycle, and according to the owner, the vaginal discharge was present for two months as dripping blood. During inspection a trickle of blood could be seen coming from the vulvar lips and palpation revealed a mass in the dorsal wall of the vagina. The dimensions were measured as approximately 3x1x1 cm. The patient had a body temperature of 39.3 oC, a respiratory rate of 32/min, a heart rate of 124 per minute, hyperaemic mucosae and normal sized lymph nodules during clinical examination. Complete blood count (CBC) values were as follows; white blood cell (WBC) 28.31 x103/mcL (Reference Interval, RI, 5-14.1 x103/mcL); red blood cell (RBC) 10.21 x106 /mcL (RI: 4.95-7.87 x106 /mcL); hemoglobin (HgB) 22.1 gr/dL (RI: 11.9-18.9 gr/dL); hematocrit (Ht) 58.59% (RI: 36-60%); lymphocyte (LYM) 8.74% (RI: 8.21%). Serum biochemistry values were; total protein (TP) 8.0 g/dL (RI: 5.4-7.5 g/dL); albumin (ALB) 2.1 g/dL (2.3-3.2 g/dL) ve globulin 5.9 g/dL (RI: 2.7-4.4 g/dL), urea 32.8 mg/dL (RI: 8-28 mg/dL) and creatinine 0.76 mg/dL (RI: 0.5-2,7 mg/dL).

A vaginal smear was taken with a cotton swab and stained with Papanicolau method, then evaluated using a light microscope. There were many lymphocytic cells with intracytoplasmic vacuoles that had a high nucleus vs cytoplasm ratio. This finding confirmed the diagnosis as TVT.

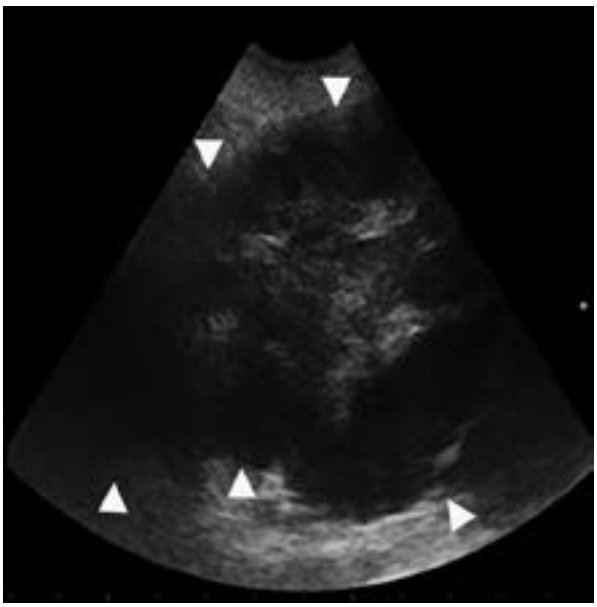

Figure 1. The ultrasonographic appearance of the mass. The mass is marked between the white arrows.

There was no discernible pathology in the patient's lateral radiograph. Ultrasonographic examination revealed no pathologies in the uterus but a mass with irregular margins including anechoic areas and having a heterogeneous structure with the dimensions of 5.9x6.5 cm was detected (Figure 1). The mass encountered during the USG examination was suspected to be a tumoral mass an ovariohysterectomy was performed.

Induction of the anaesthesia was performed with propofol (6 mg/kg, i.v. Fresenius Kabi, Sweden) and maintained by isoflurane (2%, Forane, Abbout, England) in oxygen. Meloxicam (0.2 mg/kg, i.v. Maxicam, Sanovel, Turkey) was used for pain management both 45 minutes before surgery and once daily post operatively for 2 days.

The patient was placed in dorsal recumbency and following surgical asepsis, a ventral midline incision was made caudally to provide exposure. During exploratory laparotomy all reproductive organs except the right ovary were seen in their normal anatomic positions and they had normal thickness and structure. The solid mass was at the site of the right ovary, connected to the uterus and had no adhesions to other viscera. The right ovary was not seen at the site; hence the mass was considered as an ovary tumour. After the exploration ovariohysterectomy was initiated. Initially the left suspensory ligament and adjacent broad ligament were ligated and cut. Suspensors of the right ovary could not be reached and thus incision was extended by 3 cm cranially. Same procedures were applied to the suspensors of the right ovarian mass. After releasing both horns, a transfixation ligature was place on the cervix uteri and uterine body was excised, including a portion of cervix uteri. The cervix uteri, muscles and subcutaneous tissue were closed using USP:0, PGA sutures, and the skin was closed with USP: 0, silk sutures. Later on, the vaginal mass was removed with the seemingly healthy mucosa it was attached to, and sent for histopathological evaluation to determine if the two masees were related. The vagina was sutured using USP: 2/0 PGA sutures. During the operation a Ringer Lactate

solution was given as i.v. infusion at a dosage of 10 mg/kg/hour. Following the operation, enrofloxacine (5 mg/kg/day, s.c, Baytril-K, Bayer) was administered for five days.

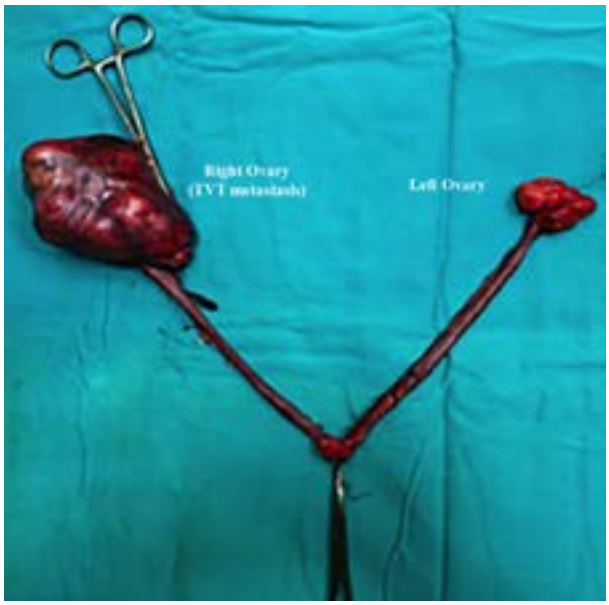

Figure 2. Postoperative image of uterine horns, right ovary and mass.

Macroscopic evaluation of the extirpated tissues were made post operatively, the right uterine horn was measured as 19.7 cm long and 0.8 cm wide, the left as 16.5 cm long and 0.7 cm wide (Figure 2). The ovarian mass was 11x9 cm in size and when cut, the cross section was white gray in colour with a multilobular arrangement showing no apparent cystic formations or fluid accumulation (Figure 3). The vaginal mass was relatively soft and had fresh blood on it.

The ovarian mass, left ovary and vaginal mass was fixed in 10% formaldehyde solution and sent for histopathological examination (Pathology department of Ondokuz Mayıs University, Faculty of Veterinary Medicine). The tissues were further dehydrated inside Histokinet (Leica, TP1020), which included alcohol and xylene series and buried in paraffin. The samples were then cut in sections of 5 microns with a microtome (Leica, RM 2125RT). The sections were dyed using hematoxylene-eozin and were later evaluated with a light microscope (Nikon, Eclipse E600).

Histopathological examination of the vaginal mass revealed widespread dispersion of uniform, round to oval cells in a fine fibrovascular stroma (Figure 4). Cells nuclei were round, with a single centrally placed nucleolus surrounded by marginated chromatin and mostly located centrally. Small amounts of light amphophilic-to-clear cytoplasm was seen in some areas where the cells are not tightly packed. Moderate rate of mitosis was observed. According to these findings, the mass was diagnosed as TVT. Because the ovarian mass (Figure 5) had the same histopathological characteristics with the vaginal mass it was presumed that the ovarian mass has developed as a result of the vaginal TVT.

The previously planned chemotherapy could not be performed because the patient owner did not bring the bitch to the clinic after the removal of sutures. Patient health status was inquired twice at 3 month intervals via phone surveys. According to this, no complications were observed following suture removal, no further drug administrations were needed and the general condition of the bitch was good.

Discussion

Transmissible venereal tumour also known as sticker sarcoma, venereal granuloma and infectious sarcoma, is still a problem in tropical and subtropical climates that have large populations of uncontrolled stray dogs. Transmission happens during mating or social activity. The transmission rate is greater in sexually active animals (Ganguly et al., 2013).

The lesion usually appears 2-3 weeks as a 1-3 mm mass after transmission and can grow to a size of 15 cm in time. Symptoms change depending on size of the mass, the effected organ or system. In genital TVT the appearance may vary but it usually appears as a cauliflower-like, fragile and bloody (Hoque, 2002). This case's age, discharge and palpation findings were in accordance to other cases in literature.

Ovarian tumours in bitches originate from epithelial cells, germ cells, sex cord stroma or mesenchymal stroma (Kennedy et al., 1998). Ovarian tumours are usually unilateral and on the left. The prevalence of ovarian tumours in bitches is uncertain because they are usually encountered during routine ovariohysterectomy or necropsy (Smith, 2003). In some papers the prevalence of the disease was reported to be between 0.5% and 1.2% (Klein, 1996). These tumours can be encountered during ultrasonographic examination although small masses may not be detected during USG (Smith, 2003).

Transmissible venereal tumour has a biological tendency to stay locally. However, metastases may occur in young, immunosuppressed or malnourished dogs (Maclachlan and Kennedy, 2008). Metastasis requires its originating tumor to be malignant, and can happen with the migration of only a few tumor cells (Cullen et al., 2008). Although it is yet uncertain how the tumour cell chooses its way or complete the process (Veer et al., 2002; Ramaswamy et al., 2003), metastasis is usually on the closer lymph nodes but sometimes far site metastases may happen (Maclachlan and Kennedy, 2008). In TVT cases, metastases to lymph nodes and other organs have been previously reported (Başţan et al., 2008; Milo and Snead, 2014).

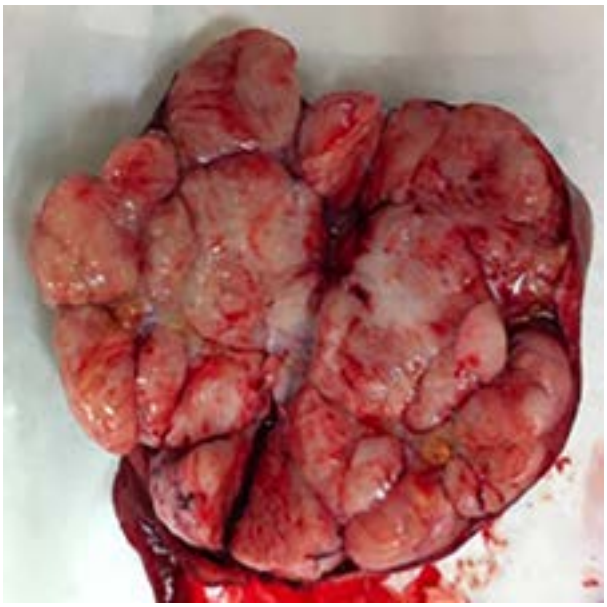

Figure 3. Sagittal section of the mass.

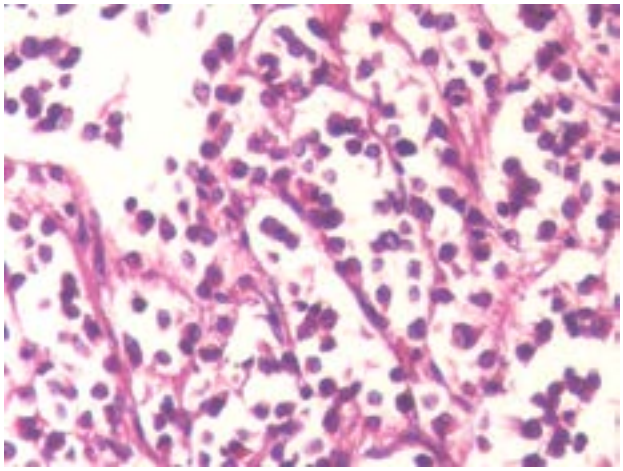

Figure 4. Uniform, round to oval cells placed on a fine fibrous stroma. Vaginal mass. TVT, Bitch, HxE, x40.

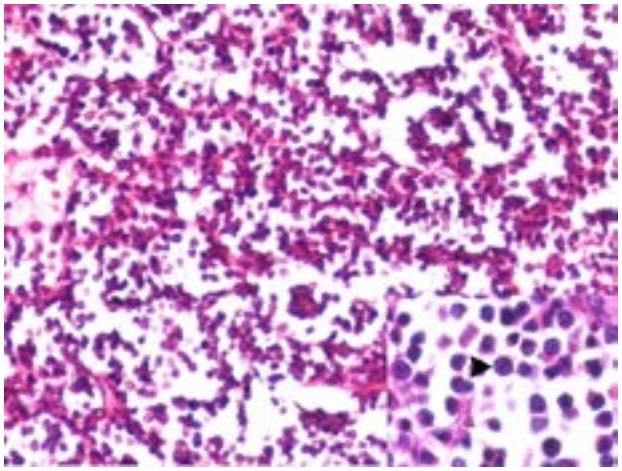

Figure 5. Histological characteristics are similar to vaginal mass. Same round to oval cells with scant amphophilic to clear cytoplasm are seen on a fine fibrous stroma. Inset: The nucleoli are big in relative to cell size and a single nucleolus placed centrally or peripherally (arrow head).Ovarian mass.TVT, Bitch. HxE, x20, Inset: x100.

The clinical findings of ovarian tumours differ depending on the type of the tumour. Symptoms like anoestrus, nymphomania, masculinisation, vaginal discharge, abdominal masses/distension (due to tumours or effusions), anorexia, vomiting, weight loss, hyperestrogenism in uterus and related pathologies such as pyometra, cystic endometrial hyperplasia (CEH) and metaplasies (Robbins, 2003) were reported. In the present clinical case clinical evaluation have revealed that the metastasis was on the right ovary and no clinical dysfunctions, discomfort or abdominal distension were seen. The patient history was incomplete due to the owner's lack of attention to the animal, so the previous cyclic activity of the bitch was unknown. Also no laboratory findigs concerning oestrogen levels were available so only the clinical findings were used to estimate the presence of hyperoestrogenism. Absence of vulvar swelling and alopecia led us to conclude that hyperoestrogenism was not present. In addition, macroscopic examination of the uterus after OHE showed there was no CEH, endometritis or metaplasia present.

The was showing advanced levels of anorexia and lethargy, however these may be attributed to the presence of chronic blood loss. Some previous TVT studies show no difference in CBC values (Das et al., 1991) while others report leucocytosis (Behera et al., 2012). The leucocytosis seen in this case was interpreted as stress related due to lymphocytosis. The increased values of RBC, HGB and HCT values combined with steady values of urea creatinine suggested primary absolute polycythemia. Large tumours are known to induce polycythaemia, increasing erythropoietin and erythropoietin extraction (Maclachlan nad Kennedy, 2008). The change to CBC in this case may be due to the metastatic mass. Low albumin count in CBC was thought to be due to an inflammatory reaction (negative acute phase proteins), the increased globulin levels were interpreted to be the result of a chronic antigenic effect. Immunologic studies concerning TVT also state that it has an antigenic effect and that effect increases as the tumour size grows (Ganguly et al., 2013). This finding may explain the increased levels of globulin in our case. However Behera et al. (2012) did not see any increase in globulin levels while there was a decrease in albumin. The retrospective study done by Kabuusu et al. (2010) show lymphopenia and mild anaemia in the CBC results.

Presence of the metastatic mass was revealed with the routine gynaecologic examination. Transmissible venereal tumour cases were also seen with uterus pathologies, ovarian remnant syndrome, abdominal/reproductive metastases and rarely urinary obstruction in previous literature which also suggests that abdominal/reproductive USG should be performed in such cases (Feldman and Nelson, 2004; Başţan et al., 2008; Sontaş et al., 2010; Chikweto et al., 2013). Healthy ovaries, especially in anoestrus, are quite difficult to detect with USG. However ovarian tumour can include solid or cystic masses. Those with cystic characteristics may be confused with non-neoplastic structures such as follicular or luteal cysts. Tumour structures are usually bigger in size and include liquid filled compartments (Smith, 2003). This data shows the accuracy of the diagnosis made with this case as an ovarian mass. In this case report, the ultrasonographic findings, the size of the tumours and sighting of the anechoic areas are similar to the aforementioned literature.

Occasionally TVT cases may recover spontaneously and most are in benign nature but all of them should receive treatment because of their progressive characteristics (Das and Das, 2000). There are many treatment methods like radiotherapy, chemotherapy, surgery, immunotherapy, cryotherapy or their combination for this purpose (Martins et al., 2005). Recently the most commonly preferred treatment choice is chemotherapy which requires weekly use of vincristine sulphate (i.v. infusion, 0.025 mg/kg) for both primary and secondary masses (Bhatia et al., 2010). However, surgery has been reported to be the first choice for ovarian tumours (Smith, 2003). In the present case, initial treatment was to continue chemotherapy using vincristine following surgical removal of the tumour mass. The resection of the vaginal mass was performed to compare the ovarian mass histopathologically to determine if metastasis was present. Resection was performed rather than a biopsy because of the small size of the vaginal mass. Unfortunately, vincristine sulphate treatment could not be carried out due to the decision of the patient owner. Surgical intervention is not recommended in TVT cases because recurrence is seen in 18-60% of cases (Ganguly et al., 2013). However, it is applicable to smaller sized masses such as in our case (Martins et al., 2005). In the bitch presented in the present study surgery seemed to be succesful for the treatment of TVT. In this case, the failure of the vaginal mass to recur later can be evaluated as the success of the surgical intervention. However, considering the high recurrence rate of surgical intervention, it can be predicted

that spontaneous recovery may play a role in remission. In TVT cases, remission starts after a two months long progressive phase14 and 16% of cases spontaneously recovery (Feldman and Nelson, 2004). Our opinion is, owing to the antigenic nature of the mass, the response of T and B-lymphocytes, TGF-B1 and NK cells might have a supportive effect during this period (Murchison, 2008).

As a result, our opinion was that sole ovarian metastases TVT are possible and could metastasize only to the ovary and a thorough clinical and gynaecologic examination; a thorough utrasonographic evaluation of the reproductive system should be performed even though the patient shows no outward clinical symptoms of any disorder.

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Ethical approval

This work involved the use of non-experimental animals only (including owned). Established internationally recognized high standards ('best practice') of individual veterinary clinical patient care were followed. Ethical approval from a committee was therefore not necessarily required.

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Review

A Review on The History of Veterinary Dentistry

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ABSTRACT

Bone and tooth infections have come across in dinosaur fossils from Mesozoic times. In the following period, tooth decays in small triple hoofed horses of the Eocene period have known definitely. The treatments that started empirically developed towards scientific methods over time and the development of veterinary dentistry was parallel to other veterinary practices.

Most of the methods and developments used in todays veterinary practice come across advenced over the centuries as basic ideas and techniques. In this review, the progress in veterinary dentistry was outlined.

Keywords: Animal dentistry, History of veterinary dentistry, History of veterinary medicine, Veterinary dental surgery, Veterinary dentistry

Veteriner Diş Hekimliği Tarihi Üzerine Bir Derleme

ÖZET

Mesozoic zamana ait dinozor fosillerinde kemik ve diş enfeksiyonlarına rastlanmaktadır. İzleyen dönemde Eozen devrinin üç tırnaklı küçük atlarında diş çürükleri kesin olarak bilinmektedir. Ampirikçe başlayan tedaviler zamanla bilimsel metotlara doğru gelişmeye başlamış ve veteriner diş hekimliği gelişimi diğer veteriner hekimliği pratiklerine paralellik göstermiştir.

Bugünkü veteriner hekimliği uygulamalarında kullanılan yöntemlerin ve gelişmelerin birçoğu temel fikirler ve teknikler olarak yüzyılların boyunca gelişmiştir. Bu derlemede veteriner diş hekimliğinin gelişimi ana hatları belirtilmiştir.

Anahtar kelimeler: Hayvan diş hekimliği, Veteriner diş cerrahisi, Veteriner diş hekimliği, Veteriner diş hekimliği tarihi, Veteriner hekimliği tarihi

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Introduction

It is known that dentistry has been intertwined with other medicine fields throughout the ages. While medicine was developing, the means of administration developed in parallel with this.

Medical historians admit that prehistoric medicine substantially uses instincts at the beginning and thereafter has a magical qualification. As we go into the depths of history, it is seen that the medical tools, which Prehistoric people are likely to use, are usually simple tools made of flint (Anon, 2003a).

In the findings of the first humans and animals, there were too many diseases, like dental caries, deformities due to rheumatism, bone tuberculosis or even similar to Syphilis. Similarly, bone and tooth infections are found in dinosaur fossils belonging to the Mesozoic period. The presence of dental caries and jaw inflammation in small three-hoof horses of the Eocene period also known. In the fossils of these horses, cracks and fractures in the jawbone have been encountered due to inflammation (Dunlop and Williams, 1996; Erk, 1966; Smithcors, 1957).

Here, it was determined that the microorganism that causes tooth decay first causes inflammation and then fractures and dislocations in the jawbone in Eocene Period (Dunlop and Williams, 1996) (Figure 1).

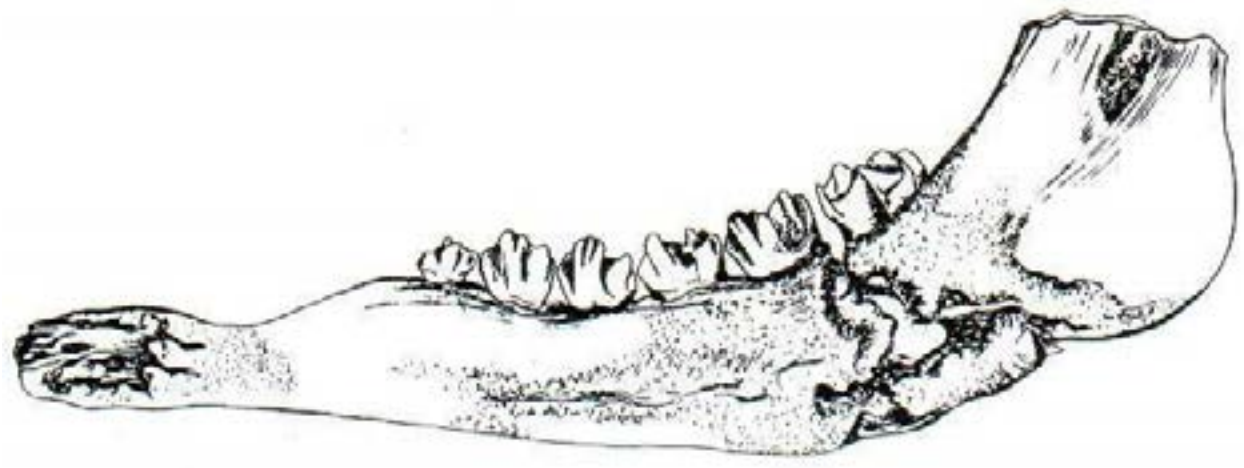

Figure 1. Fracture and dislocation of the jawbone caused by tooth decay (Dunlop and Williams, 1996).

The Emergence and Development of Veterinary Dentistry

In the development of the treatment, people started to observe the disease first on themselves, then on the animals they add into their daily lives by domesticating. Since it was impossible to find a logical cause for illnesses at that time, it was normal to think of supernatural forces as in other natural phenomena. Empirically started treatments developed towards scientific methods in time (Barbee, 1961; Dunlop and Williams, 1996; Smithcors, 1957).

Veterinary Dentistry in Ancient Times

They attached much importance to horse breeding and managing in Ancient Greece. The most important of the works on this subject is the book named "The Choice of Horses" written by Simon the Athenian and emphasizesthe need for strong teeth in horse selection (430 BC) **(**Erk, 1966**)**.

However, in this Ancient Greek scientific period, Aristotle (384-322 BC) founded natural history and comparative anatomy. A part of his book "Other Chapters of Animals" is devoted to the study of teeth, and in a book called "History of Animals", the dental systems of different animals are compared. In this description, there is a simple and faultless different type of classification of teeth according to their functions (Anon, 2002).

In the following period, Columella, one of the most well known authors in the field of agriculture and veterinary medicine in Rome, mentions the age determination of horses by teeth in his work in AD 55. The veterinarian Chiron, who lived in Byzantium in the 4th century AD, has mentioned about the teeth and jaw fractures in the 6th chapter of his book (Erk, 1966).

In the later periods (6th century AD), in "Geoponica", which contains the writings of famous physicians of the period, from the age determination with teeth was correctly mentioned. (Beckh, 1895; Dunlop and Williams, 1996).

When the time comes to the period of Islamic Civilization, Ibn Ahi Hizam, one of the famous veterinarians of the 9th century, defines "the determination of the teeth" and "the function of teeth" in the first chapter of "Book al-Hayl val-Baytara". Definition made as follows:

"Five days after the foal is born, two front teeth appear in the lower jaw, followed by two teeth in the upper jaw. These are called "Shaya". At two months of age, two more teeth appear at the top and bottom. These are called "Rubaiyat". When Foals are 8-9 months old, they have 12 teeth. The last four teeth are called "Kavarih". It is mentioned that in the book, horses use their front teeth to eat grass, and they eat barley with their molars". In this chapter, incomplete but accurate information is given about horses' teeth. Hizam says, "If there is an inflammation in the mouth due to the bridle, medicine, which is made with alcea (althaea pallida), is applied, and it is removed when outnumbering teeth are found" in chapter 9 of his book. Moreover, he mentions gingivitis and some oral and dental diseases in the 33rd episode (Erk, 1959; Erk, 1962; Erk, 1966).

In the third chapter of his most important work "Book Fadl al-Hayl" (the virtue of horses), Abd Al-Mûmin Al-Dimyâtî (1217-1306), who is another writer of Islamic civilization, mentions about that when choosing horses, their teeth were carefully looked after. Ibn Hudail, who lived in Spain in the second half of the 14th century, in the fourth part of his work titled "Hilyat al-Fursân va Şiar al-Şucan", names of various locations of horses including the teeth and in the fifth part, he mentions the qualifications that should be sought in these parts (Erk, 1962).

In the 14th century, Abu Bakr Ibn Bedr Al-Din Ibn Al-Mundhir Al-

Baytar expound the determination of age in horses by looking at teeth in the first article of his book Naserî. In addition, in the first part of the fourth article, he explains oral diseases, which are one deformity and defects of horses, and in the second part of the same article, inflammation of the gums and oral diseases (Erk, 1959). In the same period, in the middle of the 14th century, the horse physician performing the oral and dental treatment of a horse with his assistant was portrayed in the first book, of Juan Alvares de Salamiellas named (a Spanish general) "Libro de Menescalcia de Albeiteria et Fisica de las Bestias" that wrote in dedication to Al-Baytar in Spain (Dunlop and Williams, 1996) (Figure 2 and 3).

Figure 2. The horse doctor curing the horse's mouth with his assistant (Dunlop and Williams, 1996).

Figure 3. The horse doctor is doing the dental treatment of the horse (Dunlop and Williams, 1996).

In Gervase Markham's book published in 1644 under the name of "Markham's Maister - Peece", he described some applications in horses with pictures and also, illustrates the opening and fixing of a horse's mouth with *"yavaşa"*, which is a pincer made of wood or rope and attached to the lips of the horny and grumpy horses to bring them the road, for examination (Figure 4) and surgical intervention on teeth (Dunlop and Williams, 1996) (Figure 5).

Figure 4. Opening the horse's mouth in Markham's book (Dunlop and Williams, 1996).

Figure 5. Surgical intervention on horse's teeth in Markham's book (Dunlop and Williams, 1996).

In a painting made in Farrier's workshop in 1648 by the painter Paulus Potter (1625-1654), who has many drawings of animal husbandry, a veterinarian, wearing a red leather apron and taking care of the horse, and his control of teeth on the mouth of a horse under "zapt-î râpt", which is meaning of immobilizing an animal by holding or tying it up, was shown in front of the blacksmith (Flemish, 1648). Dunlop and Williams (1996), regarding this picture; "in the 17th century stated that made painting was an ordinary situation in the Netherlands but it shows the subjects applied in real life" (Figure 6).

Figure 6. Table of the veterinarian's oral and dental examination done by Paulus Potter in 1648 (Dunlop and Williams, 1996).

Veterinary Dentistry at the Beginning of the Modern Age

Anton van Leeuwenhoek discovered microorganisms in teeth and their tubular structures using a microscope in 1683 (Uzel, 1984). That's the major lines of today's veterinary medicine also were drawn in the 19th and 20th centuries (Erk, 1966). And in the diagnosis of other diseases, some developments such as penicilin, quinine, the use of X-Ray for diagnostic purposes and clinical instruments have been the pioneers of some similar diagnostic methods that will come after it in the diagnosis of dental diseases (Erk, 1966; Smithcors, 1957) (Figure 7).

Figure 7. Some clinical instruments in veterinary dentistry (Erk, 1966; Smithcors, 1957).

Veterinary dentistry will develop as a sub-component of veterinary surgery with the book "Outlines of Veterinary Medicine and Carnivor Pathology" written by Delabere Blain in 1832 (Barbee, 1961).

Meanwhile, in 1799, Sir Humphry Davy (1778-1829) suggested that nitrogen monoxide (gas that makes laugh) could be used in medical operations. Although it was also used in dentistry for a short time but was unsuccessful. William Morton (1819-1868), who worked at Boston Hospital where these unsuccessful trials were made, was advised to try ether. Later, ether was used in tooth extraction in 1846. With the discovery of anesthesia in the 19th century, important steps were taken in veterinary surgery. This development in human medicine has also made positive developments in veterinary medicine and has also been reflected in veterinary dentistry (Erk, 1966; Smithcors, 1957).

Development and Status of Veterinary Dentistry in the 20th Century

In the following periods, Dr. Lous A. Merillat published his book Animal Dentistry and Oral Diseases in 1908, the first part of which was entirely devoted to veterinary practices and animal dentistry (Merillat, 1908). Dr. Merillat defended the following hypothesis regarding the future of dentistry and veterinary medicine (Merillat, 1908):

"Although it does seem that there is no future for animal treatment in animal dentistry, it is much more accepted than is generally thought and dental operations are performed skillfully by veterinarians in veterinary dentistry as in other serious branches of surgery... The limited availability of patients, likely averted the development of animal dentistry within veterinary profession. Firstly the veterinarian, for this reason, needs the impeccability required in the practice of this art."

Merillat (1908) has compartmentalized the teeth anatomically as follows; tooth crown (head), neck, long section (molar or tooth body), and root. The terminology of Veterinary surgery has been updated with these first observations and opinions. Afterward, he has defined the teeth lost by falling out, the eruption of new ones, and the temporary teeth of dogs (milk teeth), but although not all of his thoughts about the loss of teeth and tooth eruption are true, his early writings have been effective for be recognized the importance of carnivores dental diseases.

Later, in 1925 Hobday (1925) in his book "Surgical Diseases of Dogs and Cats", described the teeth, the oral cavity, the health status of the mouth, the infection in the sinuses of carnivora, and the condition that is known as canine tooth abscess or upper 4th premolar tooth abscess (Figure 8).

Hobday (1925) states that the treatment involves the removal

of deposits, such as dental plaque or tartar from the enamel surface of the teeth. Also defined treatment as; "the instrument used to clean teeth, usually when the patient is awake and his mouth is closed, is applied under or close to the gum and scraped toward the body of the tooth; some teeth should be extracted as for part of the treatment of this disease".

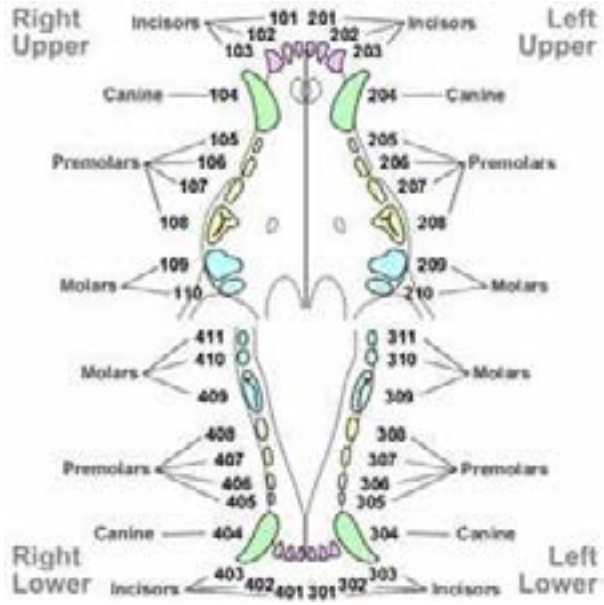

Figure 8. Jaw and tooth structure in the dog (Hobday, 1925).

Garbutt (1938), in 1938, made a theoretical definition of the condition of the teeth, formal structures, the oral cavity and dental cleaning programming according to the procedure, and dental prophylaxis in veterinary practices. He pointed to the prevention of dental plaque by brushing with salt tablet and pumice stone solutions. When he cleaned a large amount of plaque on the teeth under general anesthesia, was cleaning it along with the sick place (tissue).

Garbutt has suggested that pet owners bring the animals to the veterinarian twice a year for dental protection and treatment. This proposals, also can be named as dental prophylaxis, was the prevention, from plaque and tartar accumulation that caused tooth loss, by brushing the teeth under the supervision of a veterinarian in a veterinary clinic, cleaning and polishing with basic control with appropriate to the method (Garbut, 1938).

Garbutt (1938), again, discussed tooth loss (drop out) in dogs and suggested that he identified 42 permanent teeth-like temporary (milk) teeth, along with those outside other than temporary large molar teeth. Permanent tooth falling out problem was defined as irregularity of teeth before falling out. If temporary teeth, namely milk teeth, fall out or pull out before permanent teeth come out, it would give permanent teeth the chance to grow properly and form a regular row. However, if temporary teeth remained in the mouth, permanent teeth could erupt incorrectly. One report recommended in the treatment of this disease called malocclusion was the early separation of temporary teeth from the mouth, extirpation in mandatory situations and in the first stages of the disease (Garbut, 1938).

This practice, which has become increasingly sought after among dog breeders in recent years, is known as interceptive orthodontics. Veterinary dentistry has developed more rapidly inside of surgery, especially in the last two decades. While it continues as a sub-branch of surgical in Turkey, in some countries it is a separate branch. In some countries such as America, it has become schools that provide veterinary dentistry education under the name of Veterinary Dental College (Hale, 2003; Anon, 2003b; Anon, 2004; Anon, 2005; Wilwerding, 2001).

The development of veterinary dentistry and oral surgery shows parallelism to the development of other medical branches and veterinary practices. Many of the methods and developments used in today's veterinary practice have been shaped over centuries of duration, as basic ideas and techniques (Douglas, 1993).

Discussion, Conclusion and Inference

How did veterinary dentistry originate and develop? The answer to this question can be given accurately for the last few hundred years, and the information for previous periods is based on assumptions. As in all branches of medicine, historical examples of the development of veterinary medicine abound, and similar examples abound abundantly for veterinary dentistry, too.

As it is known, technological inventions have contributed greatly to the development of dentistry.

In other words, dentistry is one of the leading fields of medicine where technology is reflected in medicine. The necessity and benefits of knowing the histories of other fields and branches of medicine are also valid for veterinary dentistry. It is possible to explain this with the following quote that explains the benefits of knowing the history of human dentistry; *"Throughout his career, every dentist experiences a technical and technological evolution. If so, every dentist must acquire this concept (Technology Evolution Concept) so that he can apply continuous innovations in his professional life. This can be achieved by knowing the history of the profession well and understanding that medicine constantly renews itself, too"* (Anon, 2003a). *"*

Besides all these, the age of the history of this veterinary dentistry profession in Turkey and the world is still very young is another reason for why we need to know better.

It can be brought forward that this study will constitute the basis for more detailed studies on the subject.

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