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The prevalence of the enzootic bovine leukosis in cattle in Ardahan region

ABSTRACT

Enzootic bovine leucosis (EBL) is a retroviral infection which is common all over the world. EBL causes huge economic losses due to milk loss, yield loss and death. The aim of the present study was to investigate the presence of EBL by ELISA method from blood samples taken from cattle which were determined to be healthy because of clinical examination in Ardahan province and its counties in March-April 2021. For this, 500 cattle of different breeds and sexes between 1-10 years of age in different enterprises were sampled and the antibody response against EBL in the blood serum samples obtained was investigated with a commercial ELISA test kit. All 500 blood serum samples tested by ELISA were negative for EBL. In conclusion, EBL seropositivity was determined as 0% in Ardahan region as the studied area and time period.

Keywords: Ardahan, Bovine leukaemia virus, ELISA

INTRODUCTION

The Ardahan region has ideal geographical features in terms of cattle breeding with its rich meadows, pastures, grasslands and plateaus, and is among the top 10 provinces of Turkey in terms of cattle presence (Ayvazoğlu & Demir, 2020). Therefore, cattle breeding creates an important economic income for the people of the region. Both sporadic and enzootic viral infections in cattle breeding Enzootic bovine leukosis, EBL; infectious bovine rhinotracheitis, IBR; bovine viral diarrhea, BVD; blue tongue, BT; etc., causes multifaceted and great economic losses such as loss of productivity in animals, loss of product quality, Change to mortality and disease control expenses (Şimşek et al., 2017). The causative agent of the disease, BVL, is transmitted through blood (surgical procedures, infected needles, insects, etc.) (Foil et al., 1989; Van Der Maaten & Miller, 1990). Direct contamination can also be seen in intensive cattle breeding (Acar & Gür, 2013).

Enzootic bovine leukosis is a tumoral disease characterised by neoplastic cell infiltrations caused by a retrovirus called bovine leukosis virus (Bovine Leukaemia Virus, BVL) (Otlu et al., 2001). EBL emerges every year and causes great economic losses in the cattle industry due to productivity loss, loss of milk, and loss of genetic material (Gillet et al., 2007; Hooshmand et al., 2020). Cattle of all age groups are susceptible to the virus (Bordunova et al., 2021). The prevalence of the disease varies from country to country, from region to region, and even in different farms in the same region (Yıldırım et al., 2008).

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Research Article

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It has been reported that the annual prevalence of lymphoma is 1-2% in flocks infected with EBL for a long time (EFSA, 2015). Studies show that the prevalence of EBL varies between 0-59% in countries with cattle management (Savir et al., 1987; Klintevall, 1997; Zaghawa et al., 2002; Usui et al., 2003; Felmer et al., 2009; Murakami et al., 2013; de Almeida, 2021). The presence of the disease was reported in 51 countries in 2012 (EFSA, 2015). It is seen that the infection rate in small businesses varies between 0-60% in Turkey (Acar & Gür, 2013; Şimşek et al., 2017).

Enzootic bovine leukosis usually progresses clinically as asymptomatically (Chen et al., 2020; Hajj et al., 2012; Bordunova et al., 2021). It may show different symptoms depending on the organ where the infection is located. These symptoms are general symptoms such as fever, weight loss, weakness, and decreased milk yield (Yılmaz et al., 1995; Otlu et al., 2001). The significant increase in the number of circulating lymphocytes is observed in animals with tumour formation. Enlargements in lymph nodes can also be detected on rectal or vaginal palpation. The spleen, heart, kidney and uterus are among the affected organs along with lymph nodes. It should also be considered that animals have clinically neurological disorders or paralysis may have malfunctions in the brain and spinal cord (Holmes et al., 1989; Van Der Maaten & Miller, 1990; Dimmock et al., 1991; Sparling et al., 2000; Braun et al., 2007).

Agar gel immunodiffusion (AGID), radioimmunoassay (RIA) and enzyme linked immunosorbent assay (ELISA) tests are used for the diagnosis of enzootic bovine leukosis (Otlu et al., 2001). However, in recent years, the most commonly used method to perform antigen-antibody reaction is ELISA (Çoşkun et al., 2016; Acar & Gür, 2013). ELISA sensitivity and specificity was reported to be about 97.2% in a study conducted in cattle in Argentina (Trono et al., 2001).

The aim of the present study was to determine the prevalence of EBL in cattle raised in Ardahan region by ELISA method.

MATERIAL and METHOD

Animal Material

The animal material used in the study was included bovine blood serums from 500 different breeds (Simental, Brown Swiss cattle, Holstein and habitant) and gender (442 Females, 48 Males) were sampled from 38 different focal points (Table 1) belonging to Ardahan province and its districts between March and April 2021 by random sampling method. The animals used in the study were selected from clinically healthy cattle, between the ages of 1-10 and subjected to the similar care and nutrition. The blood samples taken from the vena jugularis of the cattle were centrifuged at 3000 g for 10 minutes, and were stored at -20 °C until analysis.

Detection of Antibodies against Bovine Leukaemia Virus by ELISA

BLV antibodies in bovine blood serum samples were investigated by commercial ELISA kit (Enzootic Bovine Leukosis Virus (BLV) Antibody Test Kit, Idexx, USA). The assay was performed according to the manufacturer's instructions. The absorbance values of the serum samples were measured in spectrophotometer at a wavelength of 450 nm (A (450)). The presence/absence of BLV specific antibodies in serum samples was determined by using the optical density (OD) values of the sample, with the "%S/P" formula calculated separately for each sample. The following formulas were used to calculate %S/P values.

Negative control mean:

$$\mathrm{NC}(\bar{x}) = \frac{\mathrm{NC1\ A(450)} + \mathrm{NC2\ A(450)}}{2}$$

Positive control mean:

$$\mathrm{PC}(\bar{x}) = \frac{\mathrm{PC1\ A(450)} + \mathrm{PC2\ A(450)}}{2}$$

Calculation of S/P% mean:

$$S/P\ \% = 100x\frac{\text{Sample A}(450) - \text{NC}(\bar{x})}{\text{PC}(\bar{x}) - \text{NC}(\bar{x})}$$

Evaluation:

Samples with "S/P% ≤ 60" were considered as "NEGATIVE" and samples with "S/P% > 60" were evaluated as "POSITIVE".

Statistical Analysis

Data analysis was performed using statistical software (SPSS 20.00 for windows). The data obtained were presented in the form of a table

RESULTS

The focal points and the number of animals used in the study are presented in Table 1. In the study, 351 Simmental, 122 Brown Swiss Cattle, 10 Holstein and 17 Native breed cattle were used. 156 of these animals were selected by random sampling from Ardahan Centre, 49 from Çıldır, 188 from Göle, 35 from Damal, 25 from Hanak and 47 from Posof and their affiliated villages.

Table 1. The focal points and number of animals studied in Ardahan region

Line	Focal Point	Breeds			
		Simental	Brown Swiss	Holstein	Native
1	**Centre/Centre**	17	8		5
2	**Centre/Gürçayır**	15	3	2	
3	**Centre/Sulakyurt**	9	14		3
4	**Centre/Tunçoluk**	19		3	
5	**Centre/Sugöze**	15			1
6	**Centre/Hacıali**	12	6		
7	**Centre/Hasköy**	8	3		
8	**Centre/Bağdeşen**	11			
9	**Çıldır/Centre**	10	2		2
10	**Çıldır/Öncül**	7			
11	**Çıldır/Sazlısu**	10	5		
12	**Çıldır/Âşık Şenlik**	12			1
13	**Göle/ Centre**	19	9		
14	**Göle/Demirkapı**	17	7		
15	**Göle/Sürgülen**	12	8		
16	**Göle/Tahtakıran**	14	4		
17	**Göle/Yanatlı**	11	6		
18	**Göle/Dengeli**	9	7		
19	**Göle/Dölekçayır**	11	4		
20	**Göle/Dedeşen**	17	9	3	4
21	**Göle/Molla Hasan**	13	4		
22	**Damal/Centre**	11	14		
23	**Damal/Dereköy**	10			
24	**Hanak/Centre**	13			
25	**Hanak/Binbaşak**	9	3		
26	**Posof/Aşık Zülali**	18	4	2	1
27	**Posof/Alabalık**	9			
28	**Posof/Aşık Üzeyir**	11	2		
Total		**351**	**122**	**10**	**17**

Clinical Findings

Clinical findings (enlargements of lymph nodes, parasites etc.) related to the EPL of 500 cattle enrolled in the study were normal, which were raised in Ardahan and its region and sampled within the scope of the study.

Serological Findings

The blood serum samples of 500 cattle of different races and genders collected from 28 focal points were examined by ELISA method and no antibodies were detected against BVL as a result of the analysis.

DISCUSSION

Malignant BVL, which is seen all over the world, causes systemic infections in cattle and causes great economic losses due to death, loss of productivity, treatment costs, and control/eradication programs (Otlu et al., 2001; Gillet et al., 2007; Hooshmand et al., 2020). The objective of the study was to determine the prevalence of EBL in Ardahan and its region in this study.

Studies have been carried out in many countries for the eradication of the disease and successful results have been gained (EFSA, 2015). The most appropriate way to control the disease is to eradicate sick animals (Acar & Gür, 2013). It has been reported that positivity is between 3-20% in offspring born from BVL infected cows (Ferrer & Piper 1981).

Enzootic Bovine Leukosis was reported by 51 countries and/or regions including 3 African, 6 Asian, 18 European and 21 American countries, as well as 1 region in Australia and Oceania in 2012 (OIE, 2015; EFSA, 2015). A study in Japan has been reported that BLV increased 10 times between 1980 and 2011 and reached 35.2% in a study on cattle (Murakami et al., 2013). The prevalence of the disease was found to be 59% in Chile (Felmer et al., 2009).

Studies in Turkey were found that prevalence (Tables 1 and 2). However, increases in the number of the case positive cattle in Turkey was reported (Acar & Gür, 2013). Our findings showed that the prevalence of EBL was negative in Ardahan province. There was similarity between our findings and results of the studies performed in Turkey (Otlu et al., 2001; Yıldırım et al., 2008). It was concluded that this result was caused by the closed system breeding in Ardahan and its region (the arrival of a very small amount of animals from outside and the sale of especially meat breeds outside the city) and that EBL does not pose a risk for this region.

Table 2. EBL prevalence in different studies in Turkey according to provinces

Line No	Province	Prevalence	Reference
1	Aydın	0,3%	Tan *et al.*, 2006
2	Isparta	20%	Avcı *et al.*, 2013
3	Afyon	15,45%	Acar & Gür, 2013
4	Elazığ-Malatya	2,6%	Gülaçtı *et al.*, 2004
5	Diyarbakır	1,83%	Şimşek *et al.*, 2017
6	Kars	0%	Otlu *et al.*, 2001
7	Bursa	3,06%	Şen *et al.*, 1995
8	Trakya	10,63%	Uysal *et al.*, 1995

Table 3. EBL prevalence in different studies in Turkey according to regions

Line No	Region	Prevalence	Referance
1	The Northern Anatolia Region	1,58%	Yıldırım & Burgu, 2005
2	The South Marmara Region	9,62%	Batmaz *et al.*, 1999
3	The Eastern Anatolia Region	3%	Çabalar *et al.*, 2001
4	The South-eastern Anatolia Region	0,27%	Özgünlük *et al.*, 2005

CONCLUSION

In conclusion, EBL was determined as 0% as a result of the ELISA test performed on cattle of various breeds, ages and genders raised in Ardahan and its districts. However, both sporadic and enzootic viral infections in cattle breeding cause economic losses such as loss of yield, loss of product quality, animal deaths and disease control expenses. Although the rate of EBL positivity is very low in Turkey, we think that it is important to control this infection.

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Ethical approval: The study was conducted with the decision of Ardahan University Scientific Publication and Ethics Committee dated 04.03.2021 and numbered E-29486769-622.01-1150133 and the permission of Ardahan Provincial Directorate of Agriculture and Forestry dated 09.04.2021 and numbered E-29486769-622.01-1150133

Conflict of interest: The authors declare that there is no conflict of interest for this study.

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Alteration of paraoxonase, ceruloplasmin and immunoglobulin G levels in hair goats at different ages

ABSTRACT

In this study, the alteration of paraoxonase, ceruloplasmin and immunoglobulin G levels in hair goats at different ages were investigated. 88 hair goats were included in the study. Goats were divided into five groups as 0-6 months old, 7-12 months old, 1.5-2 years old, 2.5-6 years old and 7≤ years old. Paraoxonase activity and ceruloplasmin levels were measured by spectrophotometer and immunoglobulin G was measured with ELISA kit. Although there was no statistically significant difference between the groups in paraoxonase activity, it increased until 6 years of age, but decreased with aging. A statistically significant difference was found between the groups in ceruloplasmin levels. While it provided a decrease in values up to the age of 2 and in old age, a little increase was observed in the fourth group. A statistically significant difference was found between the groups in immunoglobulin G levels, and it was observed that immunoglobulin G levels increased from newborns to adulthood, decreased with age, but increased again with aging. As a result, aging actually means an increase in reactive oxygen species, the emergence of diseases, and the loss of function of tissues and cells. Based on this, it can be said that as animals get older, the body will become open to microorganisms and the emergence of diseases will increase. These results suggest that immunoglobulin G increase with age to protect the body. Paraoxonase and ceruloplasmin levels also showed significant change with age. However, more extensive studies are needed to reveal the cause more precisely.

Keywords: Age, antioxidant, hair goats

INTRODUCTION

Goats are animals that can evaluate pasture areas and shrubs that other animals cannot. The most preferred species in the Mediterranean region is the goat because it is the most suitable animal for climate, vegetation and land structure. It is also economic to breeding, but as it harms trees and shrubs, its production and management is kept under control (Babalık and Fakir, 2007; Şirin, 2019).

Goats were domesticated around 6000 BC. It has its origins from Capra fisca (Middle European), Capra falconeri (Asia) and Capra aegagrus (Anatolia) wild goats. Goats are classified as dairy breeds, beef breeds, fiber type and combined productive breeds and fur and leather breeds. Hair Goats are one of the most grown breeds in Turkey. Except Hair Goats, Angora, Kilis, Honamlı, Norduz, Damascus, Malta and Turkish Saanen breeds are grown in Turkey too. Turkish Saanen, Malta, Damascus, Norduz and Kilis Goats are classified in dairy breeds, Angora Goats are in fiber type breeds and Hair Goats are classified in combined productive breeds (Günlü and Alaşahan, 2010; Keskin et al., 2012).

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Research Article

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In Turkey, 97% of goat assets belong to Hair Goats. It is cultivated in the Mediterranean, Aegean and Southeastern Anatolia Regions. Its bristles are generally black in color. Although their yields are generally low, they are very resistant to harsh care and feeding conditions and diseases. It is preferred because it is a breed that can easily adapt to all conditions (Keskin et al., 2012).

Paraoxonase 1 has an important role in protecting against oxidation and provides protection against lipid peroxidation. It has been demonstrated that high density lipoprotein (HDL) is associated with paraoxonase 1 and thus has antioxidative effects. Paraoxonase 1 decreases during the acute phase response and in diseases. Increase of acute phase proteins and C reactive proteins in circulation is seen with aging. Inflammatory development increases due to aging. Therefore, Paraoxonase 1 activity is thought to decrease with aging (Aviram et al., 1998). It was found that Paraoxonase 1 levels were decreased in middle-aged and elderly patients (Seres et al., 2004). Paraoxonase activity varies between animal species. It is higher in rabbits and rats than in cows, pigs and horses (Carr et al. 2015).

Ceruloplasmin is a blue colored glycoprotein containing 8 copper / molecule. It is synthesized in liver and has antioxidant properties. Ceruloplasmin is an acute phase and a transport protein. In addition to its transport function, it has many catalytic functions, from conversion of bivalent iron to trivalent iron, to the oxidation of polyamines, catecholamines and polyphenols. And because of containing copper, Ceruloplasmin is an important and highly sensitive marker for diseases associated with copper (Aliasgharpour, 2015; Merle et al., 2009).

Immunoglobulin G (IgG) is the highest level of immunoglobulin class in the blood and is produced and secreted by plasma cells in the spleen, lymph nodes, and bone marrow. Its most important function is to neutralize microorganisms and toxins. They can also take part in allergic and autoimmune reactions (Diker, 2005; Kavur et al., 2021). In colostrum, 65-90% of the immunoglobulins consist of IgG. In a study by Osaka et al. (2014), the effect of postpartum colostrum intake on serum immunoglobulin G changes in calves was shown. In the study, the effect of colostrum given immediately after birth and colostrum given after a few hours on the immunoglobulin G ratio was examined. The results showed that the earlier the colostrum is fed to the calves, the higher the serum immunoglobulin G values will be. According to these results it's important for newborns to fed them with colostrum as soon as possible.

In this study, the alteration of paraoxonase, ceruloplasmin and immunoglobulin G levels in hair goats at different ages were investigated.

MATERIAL and METHOD

Animal Groups

88 hair goats were divided into 5 groups. Group 1, 0-6 months old hair goats, group 2, 7-12 months old hair goats, group 3, 1,5-2-year-old hair goats, group 4, 2,5-6-year-old hair goats and group 5, 7≤ year old hair goats. Blood samples were collected from the jugular vein and taken in no additive tubes. After collecting, blood samples were centrifuged at 3000 xg for 10 minutes. Serum samples were stored at -86°C until analysis.

The study was carried out in accordance with animal welfare and ethical rules. Our study was carried out with the permission of the ethics committee, dated June 21, 2021, 08 meeting number and decision number 01.

Paraoxonase Test Procedure

In the paraoxon activity measurement, 50 mM glycine containing 1mM and 4 mM paraoxone; optical densities at 412 nm of p-nitrophenol formed as a result of enzymatic hydrolysis of paraoxonase using pH 10.5 buffer were

measured and paraoxonase activities were investigated (Armstrong, 2008).

Ceruloplasmin Test Procedure

Serum ceruloplasmin measurements were investigated by reading the absorbance of the colored product formed by serum samples of P-phenylene diamine dichloride (PPD) prepared in acetate buffer at pH 5.2 and 37℃, at 550 nm in the spectrophotometer (Ceron and Martinez-Subiela, 2004).

Immunoglobulin G Test Procedure

It was measured with bovine immunoglobulin G ELISA (Monoscreen® Belgium) kits. The kits were ready to use. It was read at 450 nm and after obtaining the standard curve, the values of the samples (ng/ml) were found.

Statistycal Analysis

SPSS statistical program was used for analysis. One way ANOVA test was utilized and $p<0,05$ was regarded as statistically significant. Results were submitted as Mean±Standart Error.

RESULTS

No statistically significant difference was found in the result of the paraoxonase measurement (Table 1). While the paraoxonase levels were at the lowest levels in the first and second groups, it suddenly peaked in the third group and then started to decrease again as the age progressed (Fig. 1.).

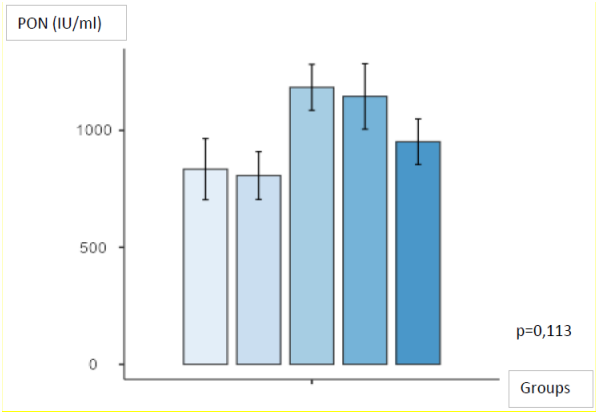

Figure 1. Serum PON activity in hair goats with different ages.

In ceruloplasmin there is a statistically significant difference between the first and second group. Groups from 2 to 5 there was found a statistically difference too (Table 1.). According to these results it can be said that there is a decrease with age progression (Fig. 2.)

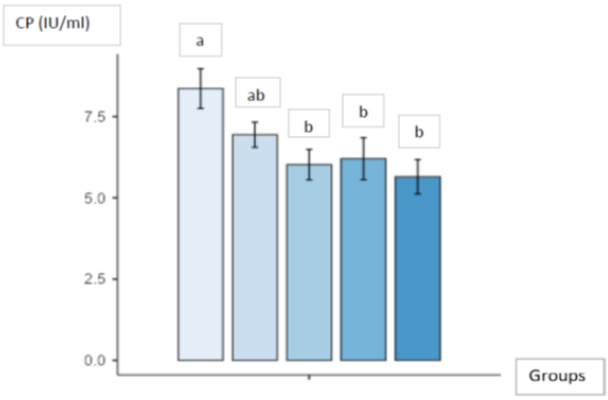

Figure 2. Serum CP levels in hair goats with different ages.

There was also a statistically significant difference between the groups in immunoglobulin G (Table 1). Second group made a sudden peak then decrease and after that it increases. Exluding the second group there is a regular increase with age (Fig. 3.).

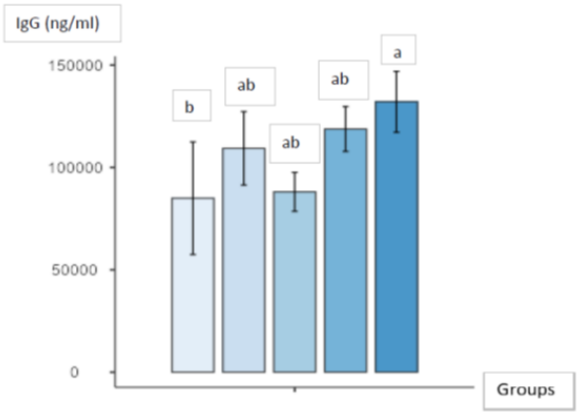

Figure 3. Serum IgG levels in hair goats with different ages.

Table 1. Serum PON, CP and IgG levels of hair goats in different age group

	Group	N	Mean±SE	p
PON (IU/ml)	0-6 months	14	834.38±130.649	0,113
	7-12 month	14	807.06±102.284	
	1.5-2 year	20	1190.63±93.412	
	2.5-6 year	20	1112.02±133.903	
	7≤ year	20	934.85±93.301	
CP (IU/ml)	0-6 months	13	8.36±0.613 [a]	0,017
	7-12 month	14	6.94±0.388 [ab]	
	1.5-2 year	20	5.78±0.505 [b]	
	2.5-6 year	20	5.78±0.505 [b]	
	7 ≤ year	19	5.78±0.505 [b]	
IgG (ng/ml)	0-6 months	14	84984±27504[b]	0,004
	7-12 month	14	109344±17932[ab]	
	1.5-2 year	19	88086±9488[ab]	
	2.5-6 year	19	118799±10941[ab]	
	7≤ year	18	132088±14871[a]	

DISCUSSION

Aging is the accumulation of destructive, progressive and universal changes that begin at birth and are responsible for increasing the risk of diseases that will cause death eventually (Halliwell and Gutteridge, 1990). It is thought that it is the aging process of the mitochondria of the cell that determines the life span. As a result of the reactions initiated by the mitochondria, free radicals are formed and mitochondria damage occurs. The rate of this damage forms the stages of aging (Harman, 1998). Electrons are attached to oxygen by mitochondrial transfer, but during this time the superoxide is accidentally separated, causing the formation of reactive oxygen species (Stadtman, 2002). Since mitochondrial DNA is more sensitive than nuclear DNA, DNA damage occurs and reactive oxygen species neutralization is reduced (Şekeroğlu, 2009).

It is known that paraoxonase is an antioxidant enzyme due to its protective effect against lipid peroxidation products (Costa et al., 2005). Paraoxonase activity may increase or decrease depending on the type of disease. In a study conducted with diabetes, it was found that paraoxonase activity increased, while paraoxonase activity decreased in cardiovasculer diseases (Martinelli et al., 2009; Savu et al., 2014). Inflammatory development increases due to aging. Therefore, paraoxonase activity is thought to decrease with aging (Aviram et al., 1998). In a study conducted by Seres et al. (2004), the change in HDL-associated paraoxonase activity by age was examined and it was found that paraoxonase levels were significantly decreased in middle aged and elderly patients compared to young people. In this study, higher paraoxonase levels are noteworthy in the young group compared to both the elderly and offspring groups. The decrease in paraoxonase levels with aging is already an expected result and has been confirmed by the studies shown. The decrease in the offspring groups can be attributed to the fact that the postnatal immune system has not yet developed as much as the young ones.

Ceruloplasmin is a positive acute phase protein and is directly related to copper metabolism. ceruloplasmin synthesis decreases in copper deficiency, while its synthesis

increases in important diseases such as inflammation, infection, diabetes, cancer and cardiovascular diseases (Tapiero et al., 2003). Aging is a biological event that causes an increase in oxidative stress and the onset of diseases, so ceruloplasmin levels are expected to increase as age increases. In this study, we see different results, in order to better understand the cause, blood copper levels in addition to ceruloplasmin should also be examined because ceruloplasmin activity decreases in copper deficiency, as copper deficiency reduces the stability of ceruloplasmin. This decrease impairs the mobilization of iron to transferrin and iron begins to accumulate in the tissues (Hellmann and Gitlin, 2002; Prohaska and Gybina, 2005). This accumulation leads to oxidative stress (Arnaud et al., 1988).

The nutrition of newborns is of great importance because the protection of colostrum is understood in line with important information such as the quality, quantity and bacterial content of the colostrum that newborns are fed (Gelsinger et al., 2015; Stott et al., 1983). It has been observed that the higher concentrations of immunoglobulin G mass the calves are fed, the longer they maintain high serum immunoglobulin G concentrations (Lopez et al., 2020). In a study by Aydoğdu et al. (2018), it was found that immunoglobulin G levels were quite different between heifers and cows. Heifers have lower imunoglobulin G levels compared to cows, due to the fact that heifers are less exposed to pathogens in the farm with age. In our study, the results showed parallelism with the studies of Aydoğdu et al. (2018) however, the reason for the sharp increase in the second group was interpreted as the stress caused by separation from the mother and feeding with concentrated food.

CONCLUSION

According to the results obtained, it can be said that there are quite significant differences. As a conclusion, no literature has been found on paraoxonase levels in healthy offsprings. This research provides a reference for paraoxonase values of healthy offsprings. Studies show that ceruloplasmin increases in diseases. As animals become prone to diseases with aging, an increase in ceruloplasmin values is expected. However, considering the relationship between ceruloplasmin and copper, copper values should also be examined in order to understand the decrease in this study more clearly. Immunoglobulin G, on the other hand, generally increased with age. Utilizing all these results, paraoxonase, ceruloplasmine and immunolgobulin G showed significant changes with age.

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Conflict of interest: The author declared no conflict of interest.

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Yetiştirici şartlarındaki Orta Anadolu Merinosu koyunlarında bazı döl verimi özellikleri

Some reproductive traits in Central Anatolian Merino sheep under breeder conditions

ÖZET

Sunulan çalışmada "Halk elinde küçükbaş hayvan ülkesel ıslahı projesi" bünyesinde yürütülen "Karaman ili Orta Anadolu Merinosu Koyun Irkının Islahı-I (70OAM2011-01)" projesinde 2017-2020 yılları arasında 21505 baş koyuna ait döl verimi özelliklerinden doğum oranı, çoğul doğum oranı, kuzu verimi, koçaltı koyun başına sütten kesilen kuzu sayısı, doğuran koyun başına kuzu verimi ve doğuran koyun başına sütten kesilen kuzu sayısı incelenmiştir. Yapılan çalışmada genel olarak doğum oranı %86.9, çoklu doğum oranı %28.99, koçaltı koyuna göre kuzu verimi 1,12 ve sütten kesim kuzu verimi 1,03, doğuran koyuna göre kuzu verimi 1,30 ve sütten kesim kuzu verimi 1,19 olarak tespit edilmiştir. Çalışmada 2017, 2018, 2019 ve 2020 yıllarında sırasıyla doğum oranı %85.3, 88.0, 86.8 ve 87.5, çoklu doğum oranı %26.47, 29.84, 34.66 ve 26.59, koçaltı koyuna göre kuzu verimi 1,08, 1,14, 1,15 ve 1,11, koçaltı koyuna göre sütten kesim kuzu verimi 0.98, 1.05, 1.07 ve 1.02 doğuran koyuna göre kuzu verimi 1.27, 1.29, 1.35 ve 1.27 ve doğuran koyuna göre sütten kesim kuzu verimi 1.14, 1.20, 1.25 ve 1.17 olarak tespit edilmiştir. Yapılan çalışmada incelen tüm özellikler yönüyle yılın önemli etkisinin olduğu tespit edilmiştir (P<0.01). Sonuç olarak Orta Anadolu Merinosu ırkı koyunlarda yapılan ıslah çalışmalarıyla zaman içinde döl verimin de artışın gerçekleşebileceği ama yıl etkisinin önemli olduğu kanısına varıldı.

Anahtar Kelimeler: Döl verimi, koyun, Orta Anadolu Merinosu, yetiştirici şartları

ABSTRACT

In the presented study, the reproductive characteristics of birth and multiple birth rates, fecundity, fecundity at weaning, litter size and litter size at weaning of 21505 sheep between 2017 and 2020 were investigated in the Project of "Breeding of the Central Anatolian Merino Sheep in Karaman-I (70OAM2011-01)" carried out under the project "Nationwide Genetic Improvement of Small Ruminants in Farm Condition". In the study, overall birth and multiple birth rates, fecundity, fecundity at weaning, litter size and litter size at weaning were 86.9%, 28.99%, 1.12, 1.03, 1.30 and 1.19, respectively. In the study, birth rate was 85.3%, 88.0%, 86.8% and 87.5%, multiple birth rate was 26.47%, 29.84%, 34.66% and 26.59%, fecundity was 1.08, 1.14, 1, 15 and 1.11, fecundity at weaning was 0.98, 1.05, 1.07 and 1.02, litter size was 1.27, 1.29, 1.35 and 1.27, and litter size at weaning was 1.14, 1.20, 1.25 and 1.17 in 2017, 2018, 2019 and 2020, respectively. In the study, the year effect was significant in terms of all the examined features (P<0.01). As a result, it has been concluded that an increase in fertility can be achieved over time with the breeding studies carried out in Central Anatolian Merino sheep, but the effect of the year is important.

Keywords: Reproduction, sheep, Central Anatolian Merino, breeder condition

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Research Article

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GİRİŞ

Koyun, genel olarak bakıldığında çok yönlü verimi, üreme kabiliyetinin yüksekliği, doğaya adaptasyonunun iyi olması, yağışı az olan bölgelerdeki zayıf otlakları bile değerlendirebilmesi, bakım-beslemesinin diğer hayvancılık faaliyetlerine göre daha kolay olması sebebiyle dünya genelinde yaygın olarak yetiştirilen bir hayvan türüdür (Kaymakçı ve Sönmez, 1996). Türkiye'de koyunculuk geniş bir coğrafyada, kırsal alanlarda yoğun bir şekilde yapılmaktadır. Hayvan varlığı olarak bakıldığında Türkiye İstatistik Kurumu (TÜİK)'nun 2021 yılı verilerine göre 42.126.781 baş koyun popülasyonuna sahiptir. Bu sayı ile Dünya'da 10. sırada yer alırken, Avrupa'da ise 1. sırada başı çekmektedir. Yetiştirilen ırk bazında değerlendirildiğinde; koyun varlığının %91.58'ini yerli ırklar, %8.42'lik kısmını ise Merinos ve Melezleri oluşturmaktadır (Şen, 2000; TÜİK, 2021).

Merinos koyunu, yapağısının kaliteli ve değerli olması nedeniyle dünya çapında en yaygın yetiştirilen koyun ırkıdır. Bunun yanında, son yıllarda birçok ülkeye benzer şekilde Türkiye'de de yapağı fiyatlarının düşük olması, Merinos koyunu yetiştiriciliğinde et verimi ve karkas özelliklerinin daha çok önem kazanmasına neden olmuştur. Türkiye'de, 1930'lu yıllardan sonra yerli koyun ırklarının Alman Et-Yapağı Merinosu koçlarla melezlenmesi sonucu et-yapağı verim yönü ağır basan yeni merinos tipi koyunlar geliştirilmiştir. Bu amaçla Alman Et-Yapağı Merinosu koçların yerli ırklarımızdan Akkaraman koyunlarıyla melezlemesi, Orta Anadolu Merinosu koyununun elde edilmesine olanak sağlamıştır (Kaymakçı ve Taşkın, 2008).

Orta Anadolu Merinosu, G2 ve G3 seviyesindeki Alman Et Merinosu x Akkaraman melezi koyun ve koçların kendi arasında çiftleştirilmeleriyle elde edilen bir ırkımızdır. Bu ırkımızda merinos genotip oranı %85'in üstündedir. Morfolojik olarak; vücut baş ve bacaklar beyaz, kuyrukları ince ve uzun, koyunlar ve koçlar boynuzsuzdur. Yapağı örtüsü bir örnek ve incedir. Akkaraman ırkına göre büyük, derin ve geniş yapılıdırlar. Canlı ağırlık, yapağı kalitesi, büyüme ve döl verimi özellikleri bakımından köken aldığı Akkaraman ırkından daha üstündür (Sönmez vd., 2009).

Orta Anadolu Merinosu ırkında Enstitü (Bahri Dağdaş Uluslararası Tarımsal Araştırma Enstitüsü) şartlarında gerçekleştirilen daha önceki çalışmalarda (Bülbül vd., 2014; Ünal, 1998; Ünal ve Akçapınar, 2001; Yalçın vd., 1972) doğum oranı, çoğul doğum oranı, kuzu verimi, koçaltı koyun başına sütten kesilen kuzu sayısı, doğuran koyun başına kuzu verimi, doğuran koyun başına sütten kesilen kuzu sayısı açısından farklı değerler elde edilmiştir.

Sunulan bu çalışmada, Karaman bölgesinde yetiştirici şartlarında yetiştirilen Orta Anadolu Merinosu koyunlarına ait bazı döl verimi özelliklerinin tespiti amaçlanmıştır.

MATERYAL VE METHOD

Araştırmanın hayvan materyalini; Tarım ve Orman Bakanlığı, Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü tarafından yürütülen Halk Elinde Ülkesel Küçükbaş Hayvan Islahı Projesi bünyesinde, Karaman İli Orta Anadolu Merinosu Islahı Projesindeki (Proje kodu: 70OAM2011-01) hayvanlar oluşturmuştur. Projedeki 25 işletmedeki 21505 baş Orta Anadolu Merinosu ırkı koyun kullanılmış ve bu koyunların yetiştirici şartlarındaki 2017-2020 yılları arasındaki 4 adet koç katımı dönemine ait verilerden döl verimi özellikleri tespit edilmiştir.

Çalışmadaki koyunlar yetiştiriciler tarafından kış dönemi haricinde meraya dayalı olarak beslenmiştir. Kış aylarında, esas olarak saman ve 100-200 gr tahıl yemi takviyeli besleme uygulanmıştır. Koyunlara gebeliğin son döneminde 200-300 gr konsantre yem ve doğumdan sonraki dönemden mera başlangıcına kadar yaklaşık 0,5-1 kg dane yem verilmiştir. Koçlar mera dönemi dışında ağılda entansif olarak beslenmiş ve üreme mevsimi boyunca normal zamanlarda anızda otlatılarak, kurak geçen yıllarda ise günde 250 g ek konsantre yemle beslenmişlerdir.

Proje kapsamındaki işletmelerde, bu bölge için koç katım sezonu olan Ağustos ve Eylül aylarında iki ay boyunca koçlar sürü içerisinde tutulmuştur. Koç katımı, sürülerde 20 baş koyun için bir baş koç olacak şekilde yapılmıştır. Koç katımı başlangıcı temel alınarak 60. günden sonra sürülerdeki koçlar uzaklaştırılmış ve işletme bazlı olarak ilk koç katım tarihinden 145 gün sonra doğum verileri toplanmaya başlanmıştır. Doğum verileri 70 gün boyunca kayıt altına alınmıştır. Alınan kayıtlardan aşağıda verilen parametreler tespit edilmiştir.

Doğum oranı (DO) (%) = Doğuran koyun sayısı/Koçaltı koyun sayısı x 100

Çoğul doğum oranı (ÇDO) (%) = Çoklu doğuran koyun sayısı/Doğuran koyun sayısı x 100

Kuzu verimi (Koçaltı koyun başına kuzu verimi (KAKBKV)) = Doğan kuzu sayısı/koçaltı koyun sayısı

Koçaltı koyun başına sütten kesilen kuzu sayısı (KAKBSKKV) = Sütten kesim yaşında yaşayan kuzu sıyısı/Koçaltı koyun sayısı

Doğuran koyun başına kuzu verimi (DKBKV) = Doğan Kuzu sayısı/Doğuran koyun sayısı

Doğuran koyun başına sütten kesilen kuzu sayısı (DKBSKKV) = Sütten kesim yaşında yaşayan kuzu sayısı/Doğuran koyun sayısı

İstatistik analiz

Elde edilen verilerden Doğum oranı ve Çoğul doğum oranına "ki kare" testi yapılmıştır. Kuzu verimi, koçaltı koyun başına sütten kesilen kuzu sayısı, doğuran koyun başına kuzu verimi, doğuran koyun başına sütten kesilen kuzu sayısına ise "ANOVA" testi uygulanmıştır. Gruplar arasındaki farklılıklar Tukey testi ile belirlenmiştir. İstatistik analizler SPSS 12 paket programı aracılıyla yapılmıştır.

BULGULAR

Sunulan çalışmada Karaman ilinde yetiştirilen Orta Anadolu Merinosu koyunlarına ait doğum oranı (DO), çoğul doğum oranı (ÇDO), kuzu verimi (KAKBKV), koçaltı koyun başına sütten kesilen kuzu sayısı (KAKBSKKV), doğuran koyun başına kuzu verimi (DKBKV), doğuran koyun başına sütten kesilen kuzu sayısı (DKBSKKV) yıllar itibariyle hesaplanarak Tablo 1'de sunulmuştur.

Tablo 1. Orta Anadolu Merinosu Koyunlarının 2017-2020 yılları arasında yetiştirici şartlarındaki bazı döl verimi özellikleri (x±Sx).

Parametreler	2017	2018	2019	2020	Genel Ortalama
n	5525	5400	5200	5380	21505
DO (%)	85.3±0.480	88.0±0.440	86.8±0.490	87.5±0.460	86.9±0.200
ÇDO (%)	26.47^{c}	29.84^{b}	34.66^{a}	26.59^{c}	28.99
KAKBKV	1.08±0.008^{c}	1.14±0.008^{ab}	1.15±0.009^{a}	1.11±0.008^{bc}	1.12±0.004
KAKBSKKV	0.98±0.009^{c}	1.05±0.009^{a}	1.07±0.100^{a}	1.02±0.009^{b}	1.03±0.005
DKBKV	1.27±0.007^{b}	1.29±0.007^{b}	1.35±0.008^{a}	1.27±0.007^{b}	1.30±0.003
DKBSKKV	1.14±0.009^{c}	1.20±0.009^{a}	1.25±0.010^{a}	1.17±0.009^{b}	1.19±0.005

a,b,c: Aynı satırda farklı harf taşıyan değerler arasındaki fark istatistiki açıdan önemlidir (P<0.01)
DO: Doğum oranı, ÇDO: Çoğul doğum oranı, KAKBKV: Kuzu verimi, KAKBSKKV: Koçaltı koyun başına sütten kesilen kuzu sayısı, DKBKV: Doğuran koyun başına kuzu verimi, DKBSKKV: Doğuran koyun başına sütten kesilen kuzu sayısı.

TARTIŞMA

Orta Anadolu Merinoslarında halk elinde yürütülen bu çalışmada doğum oranı haricinde kalan diğer incelenen bütün parametrelerde yıllar arasında istatistiki fark tespit edilmiştir (P<0.01). Non genetik parametrelerin koyunlarda reprodüktif parametreler üzerine etkisi farklı ırklar üzerinde gerçekleştirilen önceki çalışmalarda vurgulanmıştır (Gbangboche vd., 2006; Mandakmale vd., 2013; Safari vd., 2013). Dolayısıyla diğer birçok çalışmada (Dixit vd., 2001; Rosa ve Bryant, 2003; van Wyk vd., 2003) belirtildiği gibi sunulan çalışmada da non genetik parametrelerden yılın belirtilen reprodüktif verim parametreleri üzerine etkisi önemli bulunmuştur.

Yıllar arasındaki farklar incelendiğinde çalışmanın yapıldığı ilk yıla göre bir artışın olduğu gözlenmektedir. Bunun en büyük sebebinin bazı araştırmacıların (Buckrell, 1987; Notter, 2012) bildirdiği üzere çalışmada hayvan ıslah koşullarının sağlanmaya başlanması, damızlık seçimi, sürülerde etkin ayıklamanın yapılması ve sürü yönetiminin daha iyi uygulanması olabileceği düşünülmektedir. Bunun yanında 2020 yılında gözlenen reprodüktif verim düşüşünün yukarıdaki literatür bildirişlerde belirtildiği üzere yıl bazında çevresel faktörlere bağlı değişimden kaynaklanabileceği değerlendirilmektedir.

Reprodüktif verim özellikleri, koyun yetiştiriciliğinde karlılığı etkileyen ana faktörler olarak kabul edilmektedir (Mokhtari vd., 2010). Sunulan çalışmada; halk elinde yetiştirilen sürülerden elde edilen doğum oranının (yıllara göre %85-88 arası, ortalama %86.9), Enstitü şartlarında aynı ırkla çalışan Yalçın vd. (1972), Ünal ve Akçapınar (2001) ve Bülbül vd. (2014)'nin elde ettikleri değerlerin (sırasıyla %80.8, 87.8 ve 90.5) arasında olduğu tespit edilmiştir. Çoğul doğum oranı ise sunulan çalışmada yıllara göre %26,47 ile 34,66 arasında (ortalama %28,99) gerçekleşirken Ünal (1998)'ın %39.6 olarak bildirdiği değerden düşük bulunmuştur.

Çalışmada saptanan kuzu verimi ortalaması 1.12 (yıllara göre 1.08 ile 1.15 arasında) Bülbül vd., (2014)'nin tespit ettiği değerden (1.10) yüksek olurken, Ünal ve Akçapınar (2001)'ın çalışmasıyla (1.13) benzer, Yalçın vd. (1972)'nin bildirdikleri değerden (1.27) ise daha düşük olarak belirlenmiştir. Koçaltı koyun başına sütten kesilen kuzu sayısı açısından değerlendirildiğinde sunulan çalışmada elde edilen ortalama değer (yıllara göre 0.98-1.07, ortalama 1.03) daha önce bu konuda Enstitüde çalışan Ünal ve Akçapınar (2001) ve Bülbül vd. (2014)'nin bildirdikleriyle benzer olarak gerçekleşmiştir.

Sunulan çalışmada doğuran koyun başına kuzu verimi yıllara göre 1.27 ile 1.35 arasında değişmekle birlikte ortalama 1.30 olarak tespit edilmiş ve elde edilen bu ortalama değer Bülbül ve ark (2014) ve Ünal ve Akçapınar (2001)'ın bildirdikleri değerlerin (sırasıyla 1.24 ve 1.40) arasında bulunmuştur. Çalışmada saptanan doğuran koyun başına sütten kesilen kuzu sayısının ise (yıllara göre 1.14 ile 1.25 arasında) ortalama 1.19 değeri ile Bülbül vd. (2014) ve Ünal ve Akçapınar (2001)'ın çalışmalarında belirttikleri değerlerle (sırasıyla 1.14 ve 1.23) benzer olduğu tespit edilmiştir.

Bu çalışmada elde edilen değerlerle diğer çalışmaların değerleri arasındaki farklılıkların en önemli nedenin; bu çalışmada 25 farklı işletmedeki 4 yıllık verinin kullanılmasına karşın diğer çalışmaların tek bir işletmede yürütülmüş olmasından kaynaklanabileceği düşülmektedir. Bunun yanında çalışmaların farklı yıl, iklim, çevre, bakım ve besleme şartları altında yürütülmelerinin de elde edilen sonuçlar üzerinde etkisi olabilir.

SONUÇ

Sonuç olarak Karaman bölgesinde yetiştirici koşullarında yetiştirilen Orta Anadolu Merinosu ırkı koyunların verimlerinin büyük oranda diğer çalışmalarla benzer olduğu ve bu ırkın üzerinde yapılan ıslah çalışmaları ile zamanla reprodüktif parametrelerde bir artış sağlanabileceği, ancak yılın etkisinin önemli olduğu sonucuna varılmış. Bu nedenle hayvan verimliliğini artırmaya yönelik ıslah çalışmalarının devam etmesi gerektiği düşünülmektedir.

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Etik beyan: Sunulan çalışma bünyesinde hayvanlara herhangi bir müdahalede bulunulmamış, çalışmada kullanılan veriler hayvan sahiplerinin rutin hayvan yetiştirme uygulamaları neticesinde derledikleri verilerden analiz edilmiştir. Bu anlamda sunulan çalışma için Etik kurul onayı gerekmediğini çalışmanın yazarları olarak beyan ederiz.

Çıkar çatışması: Yazarlar, bu makale için çıkar çatışması olmadığını beyan eder.

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Marmara bölgesinde ruminantlardaki abort olgularında leptospirozisin levaditi ve immunohistokimyasal yöntemlerle teşhisi ve histopatolojik bulgularla karşılaştırılması

Diagnosis of leptospirosis with levaditi and immunohistochemical methods in abortion cases in ruminants in Marmara region and comparison with histopathological findings

ÖZET

Leptospiroz, abort, ölü doğum, kısırlık ve süt verimi kayıplarına neden olan ve hayvancılık için ekonomik açıdan çok önemli, zoonoz karakterli, bakteriyel bir enfeksiyondur. Bu çalışmada, 2013-2018 yılları arasında Marmara Bölgesi'ndeki il ve ilçelerden Pendik Veteriner Kontrol Enstitüsüne getirilen ruminantlardaki abort olgularından leptospirozun immunhistokimyasal (İHK) ve Levaditi yöntemlerle teşhisi ve histopatolojik bulgularla karşılaştırılması amaçlanmıştır. Materyal olarak 12 farklı ilden 750 kuzu, 218 oğlak ve 284 buzağı olmak üzere toplam 1252 adet abort fötüs kullanılmıştır. Fötüslerin nekropsileri yapıldıktan sonra iç organlardan alınan örnekler rutin takipleri yapılarak, hematoksilen eozinle boyanmıştır. Ayrıca böbrekler, karaciğer ve akciğerlerden İHK ve Levaditi yöntemleriyle leptospira etkenleri aranmıştır. Çalışmada, makroskobik olarak bazı fötüslerde deri altı ödemleri ve vücut boşluklarında sıvı birikimi, ikterus, karaciğerde miliyer nekroz odakları görülürken, çoğunda otolitik değişiklikler izlendi. Mikroskobik incelemelerde karaciğerde periasiner nekroz ve böbrek tubullerinde nekroz ve hiyalin silindirlerine rastlandı. İHK yöntemle yapılan boyamalarda 1252 adet fötusun 160 tanesinde ve İHK pozitif örneklerin Levaditi yöntemle boyamalarında ise 108 adet pozitif boyanma tespit edildi. Levaditi boyama metodu ile etkenler sadece böbrekte belirlenirken, karaciğer ve akciğerde gözlenmedi. Ancak İHK metodu ile karaciğer, böbrek ve akciğerde de pozitif boyanmalar saptandı. Sonuç olarak Marmara Bölgesi'ndeki ruminantlarda leptospiroza bağlı abort oranı %12,77 olarak belirlenmiştir. Leptospirozun teşhisinde, İHK metodunun Levaditi boyama yöntemine göre daha hassas olduğu gösterilmiştir. Çalışma sonucunda Marmara Bölgesi için ruminantlarda leptospirozun önemli bir abort sebebi olduğu ortaya konulmuş, hastalıkla ilgili farkındalığın arttırılması ve aşılamaların yapılmasının hastalıkla mücadelede çok etkin olacağı sonucuna varılmıştır.

Anahtar Kelimeler: Abort, immunohistokimya, leptospiroz, Levaditi, ruminant.

ABSTRACT

Leptospirosis is a bacterial infection of zoonotic character, which causes abortion, stillbirth, infertility and milk yield losses and is very important for livestock economically. In this study was aimed to diagnose and compare the abortion cases with leptospirosis in ruminants coming to Pendik Veterinary Control Institute from the provinces and districts of Marmara region between 2013-2018 by immunohistochemical (IHC) and Levaditi methods and to compare with histopathological findings. A total of 1252 aborted fetuses; 750 lambs, 218 kids and 284 calves from 12 different provinces were used as materials. After the necropsies of the fetuses, the samples taken from the internal organs were routinely followed and stained with hematoxylin eosin. In addition, leptospira agents were searched by IHC and Levaditi methods from kidneys, liver and lungs.

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Research Article

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In the study, macroscopically, some fetuses showed subcutaneous edema and fluid accumulation in body cavities, icterus and miliary necrosis foci in the liver, but most had autolytic changes. Microscopic examination revealed periaciner necrosis in liver and necrosis and hyaline cylinders in renal tubules. Positive staining was observed in 160 of 1252 fetus samples in IHC staining and Levaditi staining of IHC positive samples revealed 108 positive staining. Levaditi staining method was used to determine the agent only in kidney tissue, but not in the liver and lung. However, positive results were obtained in liver, kidney and lung by IHC method. As a result, the abortion rate of leptospirosis in ruminants was determined as 12,77% in Marmara region of Turkey. In the diagnosis of leptospirosis, IHC method is more sensitive than Levaditi staining method. As a result of this study, it has been shown that leptospirosis has an important place in abortions in ruminants in Marmara Region of Turkey and it is concluded that raising awareness about the disease and vaccination will be very effective in combating the disease.

Keywords: Abortion, immunohistochemistry, leptospirosis, Levaditi, ruminant.

İRİŞ

Leptospiroz, abort, ölü doğum, kısırlık ve süt verimi kayıplarına neden olan, hayvancılık için ekonomik açıdan çok önemli, zoonoz karakterli, bakteriyel bir enfeksiyondur (Bolin, 2005). Hastalık akut septisemik veya kronik nefritik formlarında gözlenir. Etken hastalığı geçiren hayvanların böbreklerinde mikrokoloniler halinde lokalize olur ve uzun süre idrarla atılır (Türkütanıt vd., 2002; Villanueva vd., 2016).

Patojenik leptospira türleri, yabani ve evcil birçok memeli türü tarafından taşınır. Direkt veya indirekt yollarla diğer hayvanlara bulaştırılır (Villanueva vd., 2016). Hastalığa ilgili oluşan üreme sorunları ve süt üretiminde düşüşler nedeniyle sığır ve koyun endüstrisinde ekonomik kayıplara yol açar. Ayrıca enfekte hayvanlarda kronik renal enfeksiyon gelişir. Enfekte hayvanlar idrarları ile etkeni diğer hayvanlara yayarak hayvansal üretim ve ilgili endüstrilerde çalışanlar için potansiyel zoonotik bir tehdit oluşturur (Fang vd., 2014). İkterus, hemoglobinuri, organ ve dokularda kanamalar, abortus, mastitis ve septisemi ile seyreder. Olguların %25-30'unda abortus vakası görülür. *Leptospira (L.) hardjo* ve *L. pomana* abortus ve infertilitenin önemli serovarlarıdır. Etkenler uzun süre böbrek ve genital sistemde canlı kalabilirler. Etken fötusa plasenta yolu ile geçer. Hayvanlar arasında bulaşma çiftleşme, temas ya da kontamine çevre ile olmaktadır. Genellikle gebeliğin son üçte birlik döneminde abortus şekillenir. Fötus otolize uğramış şekilde atılır (Özdemir ve Erer, 2018). Leptospiroz, koyun ve sığırlarda benzer semptomlar gösterir. Hastalık ateş, anoreksi, şiddetli sarılık, hemoglobinüri, anemi, sinirsel belirtiler ve ölüm ile karakterizedir. Gebeliğin son üçte birinde abort, zayıf yavru doğumu, ölü doğum ve kısırlık gibi üreme sorunları,akut leptospirozda koyunlarda, ateş, apati, dyspnea, septisemi, zayıflık ve ölüm ortaya çıkar (Lucheis ve Ferreira, 2011).

Hastalığın teşhisi etken izolasyonu, serolojik ve histopatolojik muayenelerle yapılır. Ancak son yıllarda immunperoksidaz tekniği ile formolde tespit edilip parafinde bloklanan doku kesitlerinde bakteriyel antijenin saptanmasıyla tanıya gidilmektedir (Temur ve Sağlam, 2003). Leptospirozun laboratuvar tanısı, klinik örneklerin doğrudan incelenmesine, etkenin izole edilmesine, serolojik testler yoluyla anti-leptospiral antikorların tespit edilmesine veya etken DNA'sının moleküler yöntemlerle tespit edilmesine dayanır (Bisias vd., 2009, Fang vd., 2014). Kültür, çeşitli örneklerden bakteri izole edilmesi nedeniyle önemli tanımlayıcı bir teşhistir. Ancak uzun zaman alması, zahmetli olması ve diğer etkenlerle kontamine olması nedeniyle zordur. (Fang ve ark. 2014). Polimeraz zincir reaksiyonu (PZR) analizleri, 1990'lardan bu yana leptospirozun tanısında güvenilir ve hızlı moleküler yöntemler olarak kullanılmaktadır (Fang vd., 2014; Lucheis ve Ferreira, 2011). Levaditi boyamada, sarı zemin üzerinde siyah boyanmış spiral etkenler görülür.

Etkenin iyi boyanabilmesi için dokunun tespit edilmeden önceki tazeliği büyük önem arz etmektedir. Aksi durumlarda etkenin görünümünde değişimler olduğu belirtilmiştir (Falini ve Taylor, 1983; Russell ve Faine, 1984).

Sunulan çalışmada, Pendik Veteriner Kontrol Enstitüsü'ne (PVKE) Marmara Bölgesi'ndeki illerden 2013-2018 yılları arasında getirilen ruminantlara ait abort olguları içerisinde leptospirozun teşhisinde kullanılan Levaditi ve immuhistokimyasal (İHK) yöntemlerinin karşılaştırması ile bu olgularda gözlenen histopatolojik değişikliklerle uygunluğu araştırılarak teşhiste hangi yöntemlerin güvenli olarak kullanılabileceği amaçlanmıştır.

MATERYAL VE METHOD

Bu çalışmada, Marmara Bölgesi'nin farklı il ve ilçelerinden 2013-2018 yılları arasında Pendik Veteriner Kontrol Enstitüsü'ne gönderilen 1252 adet sebebi bilinmeyen veya leptospiroz şüpheli aborte fötuslerin (koyun fötus, keçi fötus, sığır fötus) nekropsileri yapılarak doku örneklerinin incelemeleri yapıldı.

Histopatolojik inceleme

Soğuk zincir şartlarında PVKE'ne kargoyla veya elden getirilen fötuslere ait örnekler, patolojik incelemeler için %10'luk tamponlu formalinde tespit edildikten sonra rutin laboratuvar metotları ile hazırlanan parafin bloklardan mikrotomda 5 mikron kalınlığında kesitler alınarak tümü Hematoksilen&Eozin (H&E) ile boyandıktan sonra (Luna, 1968) ışık mikroskobunda incelendi.

Levaditi Boyama

Formol tespitini takiben 2 mm inceliğinde trimlenen doku örnekleri, çeşme suyunda 1-3 saat süreyle yıkandı. 24 saat süreyle % 96'lık alkolde tutuldu. Doku, kabın dibine çökünceye kadar distile suda tutuldu. Taze hazırlanmış gümüş nitrat solüsyonu (%3) içine konuldu ve karanlıkta 37 ^{o}C'de 3-5 gün solüsyon yenilenerek bekletildi. Distile suda çalkalandı. Karanlıkta oda sıcaklığında, 1-3 gün indirgeme solüsyonunda bekletildi. Distile suda çalkalandı. %80, %96'lık alkol ve absolut alkollerden her birinde 30 dakika (dk) ve 2 ksilol kabında 1'er saat tutularak geçirildi ve 2 parafin kabında 45'er dk bekletildi. Parafine gömüldükten sonra 5 µm kalınlığında alınan doku kesitleri, ksilollerden geçirilerek üzerleri lamelle kapatıldı.

İmmunhistokimyasal Boyama

İHK boyamalar BioSite Histo HRP Kit (Novex, BioSite, KDB-10016) prosedürüne göre yapıldı. Lizinli lama alınan kesitler 1 gece boyunca 37°C'de veya 60 °C'de 1 saat inkube edildi. İnkubasyonu tamamlanan kesitler 3 kez 5'er dk ksilolde tutuldu. Alkol serilerinden 2 kez 10'ar dk geçirildi. Antijen retrieval için sitratlı tamponda (pH 6.0) mikrodalgada 800 watta 15 dk kaynatıldı. Bundan sonra aynı tampon içerisinde 400 watta 15 dk mikrodalgada bekletildi. Sonra 20 dk dış ortamda bekletildi. % 3'lük hidrojen peroksitte 20 dk tutuldu (3 cc hidrojen peroksitt 97 cc methanol). Sonra preperatları başka bir kaba alıp Fosfat tampon çözeltisi (PBS, pH 7,6) 10 dk bekleyerek yıkama yapıldı. Karanlık ortamda PBS'den çıkarıp preperatın altını ve etrafını kuruladıktan sonra Protein Bloklama Çözeltisi (blocking solution) damlatıldı ve 10 dk bekletildi. Preperatın üzerindeki fazla blocking solution kuruladıktan ve fazlalığı dökdükten sonra üzerine Rabbit Anti Leptospira Antibody (Invitrogen, PA1-7228) döküldü ve 1 saat karanlık ortamda bekletildi. 2 kez 5'er dk PBS ile yıkandı. Etrafını kurulayıp Broad Spectrum Seconder Antibody damlatıldı. 10 dk karanlık ortamda bekletildi. Tekrar 10 dk PBS yıkama kurulama aşamasından sonra HRP Streptavidin karanlık ortamda 10 dk tutuldu. Sonra tekrar 10 dk PBS ile yıkandı. Kromojen olarak (karanlık ortamda her preparat için 200 µl) diamiobenzidine (DAB) (ScyTek, 38607) damlatıldı ve 10 dk beklendi. Distile suda yıkandıktan sonra Mayer Hematoksilende 3-5 dk bekletilip, suda mavileşinceye kadar yıkandı. Alkol–Ksilol serilerinden geçirilip entellan ile kapatıldı.

BULGULAR

Çalışmada PVKE'ne 2013-2018 yılları arasında gelen toplam 1252 abort örneğinden İHK yöntemi ile 160 olguda (%12,77) leptospira pozitifliği belirlenmiş olup yıllara göre dağılımı Tablo 1'de verilmiştir. İncelenen 750 koyun fötusünün 76'sında (%10,13), 218 keçi fötusunun 26'sında (%11,92) ve 284 sığır fötusunun 58'inde (%20,42) İHK yöntemiyle pozitiflik saptanmıştır (Tablo 2).

Tablo 1. Örneklerin yıllara göre toplam ve İHK pozitif sayıları

Yıl	Örnek Sayısı	İHK Pozitif Örnek Sayısı
2013	217	42
2014	153	38
2015	190	27
2016	190	22
2017	264	22
2018	238	9
Toplam	**1252**	**160**

Tablo 2. Örneklerin ruminant türü, İHK pozitif sayı ve oranları.

Ruminant türü	İncelenen fötüs sayısı (n)	İHK Pozitif fötüs sayısı (n) ve oranı (%)
Koyun	750	76 (%10,13)
Keçi	218	26 (%11,92)
Sığır	284	58 (%20,42)
Toplam	**1252**	**160 (%12,77)**

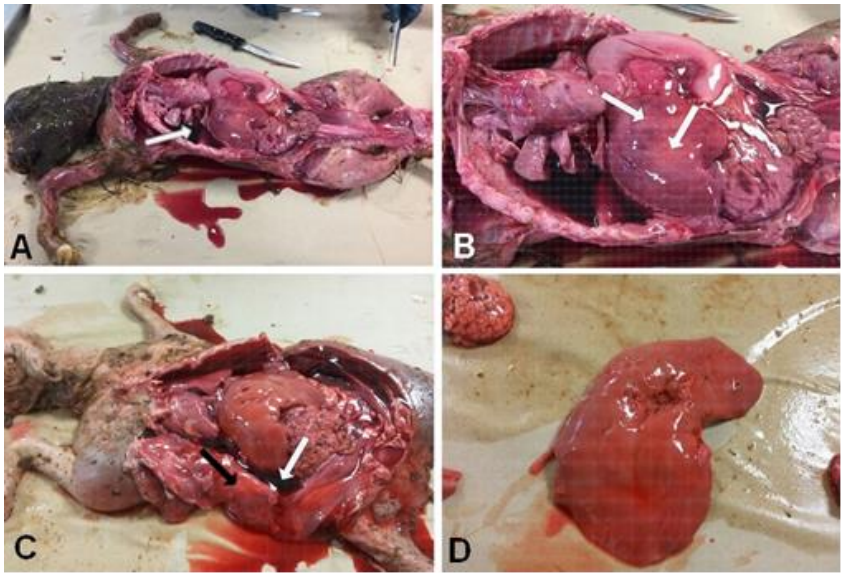
Çalışma sonucunda elde edilen pozitif fötus örneklerinin Marmara Bölgesi'ndeki illere göre dağılımı Tablo 3'de verilmiştir.

Tablo 3. Örneklerin yıllara ve Marmara Bölgesi'ndeki illere göre dağılımı.

		2013			2014			2015			2016			2017			2018			
		Koyun	Keçi	Sığır	Koyun	Keçi	Sığır	Koyun	Keçi	Sığır	Koyun	Keçi	Sığır	Koyun	Keçi	Sığır	Koyun	Keçi	Sığır	TOPLAM
Kırklareli	**Örnek**	15	5	12	5	3	3	14	8	5	13	5	7	19	12	10	23	8	6	**173**
	Pozitif	1	1	2	1	1	1	3	2	1	1	1	2	1	1	3	1	-	-	**23**
Edirne	**Örnek**	23	9	11	9	6	4	18	7	7	16	7	7	21	8	9	26	4	3	**195**
	Pozitif	4	3	5	3	3	2	1	-	2	2	1	3	2	-	3	1	-	-	**35**
Tekirdağ	**Örnek**	14	6	4	5	1	3	12	4	3	14	3	5	17	5	5	17	1	3	**122**
	Pozitif	4	2	1	-	-	1	-	-	1	-	-	1	-	1	1	-	-	-	**12**
Kocaeli	**Örnek**	9	-	6	9	3	5	9	1	7	13	6	5	13	2	3	12	1	3	**107**
	Pozitif	2	-	1	3	-	1	1	-	1	1	-	1	1	-	-	-	-	-	**12**
Yalova	**Örnek**	4	1	2	5	3	2	4	-	-	4	2	2	5	-	-	5	-	1	**40**
	Pozitif	1	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	**3**
Sakarya	**Örnek**	9	-	3	5	1	5	2	-	-	7	-	-	9	-	5	11	2	-	**59**
	Pozitif	1	-	-	1	-	2	-	-	-	-	-	-	-	-	-	-	-	-	**4**
Bursa	**Örnek**	8	3	-	8	1	5	9	2	8	9	4	8	18	5	3	17	4	8	**120**
	Pozitif	1	1	-	1	-	1	1	-	1	1	-	1	1	-	1	1	-	-	**11**
Bilecik	**Örnek**	8	4	3	6	-	3	5	-	3	2	-	2	11	2	3	8	2	1	**64**
	Pozitif	-	-	-	1	-	1	-	-	2	-	-	1	1	-	-	3	-	-	**9**
Balıkesir	**Örnek**	15	7	11	18	7	5	19	8	6	14	2	8	23	5	6	26	6	7	**193**
	Pozitif	3	2	3	4	1	2	2	3	2	2	-	2	1	1	2	-	-	-	**30**
Çanakkale	**Örnek**	8	5	3	9	3	2	8	4	5	9	4	5	14	7	5	17	1	1	**110**
	Pozitif	1	-	-	4	1	1	1	-	1	1	-	-	-	1	-	3	-	-	**14**
İstanbul	**Örnek**	2	-	-	3	-	-	1	-	-	-	-	-	2	-	-	-	-	-	**8**
	Pozitif	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0**
Düzce	**Örnek**	5	1	1	3	-	3	5	1	4	4	2	1	12	2	3	8	2	4	**61**
	Pozitif	2	-	-	1	-	1	1	-	-	1	-	-	1	-	-	-	-	-	**7**

Makroskobik Bulgular

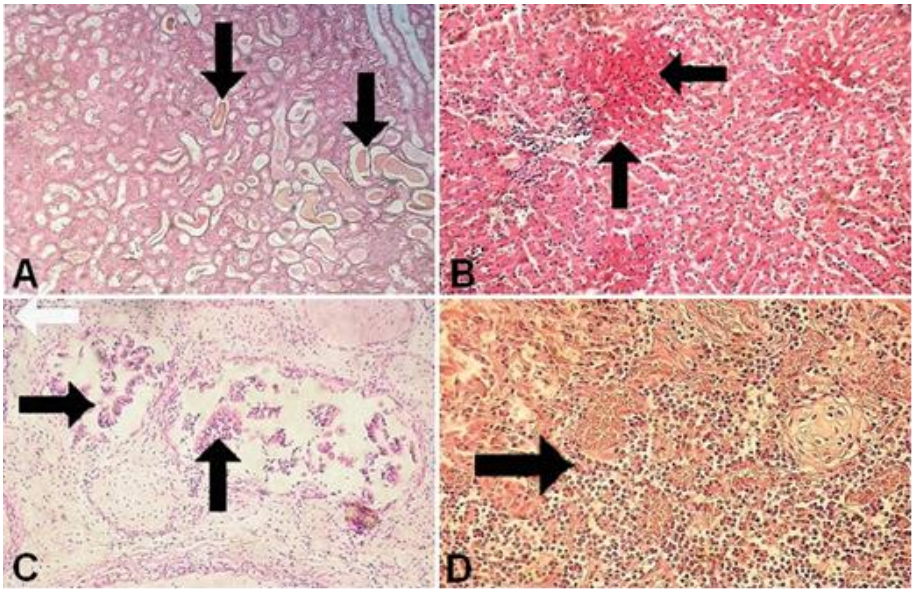
Nekropsisi yapılan ruminant fötuslarının bazılarında vücut boşluklarında kanlı eksudat, deri altı ödem (Şekil 1A-C), böbreklerde şişkinlik ve peteşiyel kanamalar, karaciğerde nekroz odakları ve otolitik değişiklikler (Şekil 1C,D) dikkati çekti.



Şekil 1. A. Sığır fötusun vücut boşluğunda kanlı eksudat (ok), B. Karaciğerde nekroz odakları (oklar), C. Aborte kuzuda otolitik değişiklikler. Deri altında biriken ödem (siyah ok) ve kanlı eksudat (beyaz ok), D. Karaciğer solgun, yumuşak ve büyümüş. (PVKE Patoloji Lab. Arşivi)

Histopatolojik Bulgular

Çalışılan dokulardan hazırlanan doku örneklerinin histopatolojik incelemelerinde en belirgin değişiklikler sırasıyla böbrek, karaciğer ve akciğerde gözlendi. Böbreklerin tubulus lümenlerinde hiyalin silindirleri (Şekil 2A), tubulus epitellerinde nekroz, interstisyumda yaygın kanama alanları ve nötrofil lökosit infiltrasyonları tespit edildi. Karaciğerde periasiner nekroz (Şekil 2B) ve hepatositlerde dejenerasyon saptandı. Akciğerlerde bronş ve alveol epitellerinde dökülme (Şekil 2C) ile lümenlerinde nötrofil lökosit infiltrasyonu gözlendi (Şekil 2D).



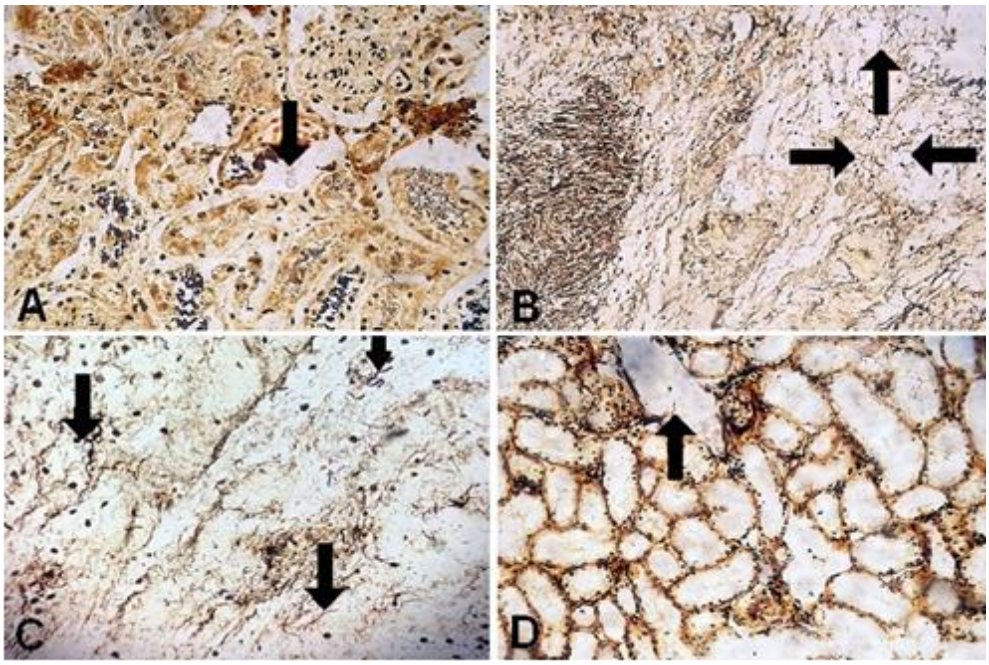
Şekil 2. A. Böbrekte medulladaki tubul lümenlerinde hiyalin silindirleri (oklar), H&E, X200, B. Karaciğerde periasiner nekroz (oklar) ve hepatositlerde dejenerasyon, H&E, X200, C. Akciğerde bronş epitellerinde dökülme (siyah oklar) ve bronş çevresinde nötrofil lökosit infiltrasyonu (beyaz ok), H&E, X200, D. Akciğerde alveoller içerisinde nötrofil lökosit infiltrasyonu (ok), H&E, X400.

Levaditi Bulgular

Gümüşleme (Levaditi boyama) metoduyla 108 örnekte ve sadece böbrek dokusunda pozitiflik tespit edildi. Boyanmalar böbrek tubul epitellerinde ve lümenlerinde sarı zeminde siyah renkte boyanmış spiral etkenler olarak görüldü (Şekil 3 A-D). İHK ve Levaditi boyanma sonuçları Tablo 4'de verildi.

Tablo 4. İHK ve Levaditi yöntemlerinde organlara göre pozitif sonuçların dağılımı.

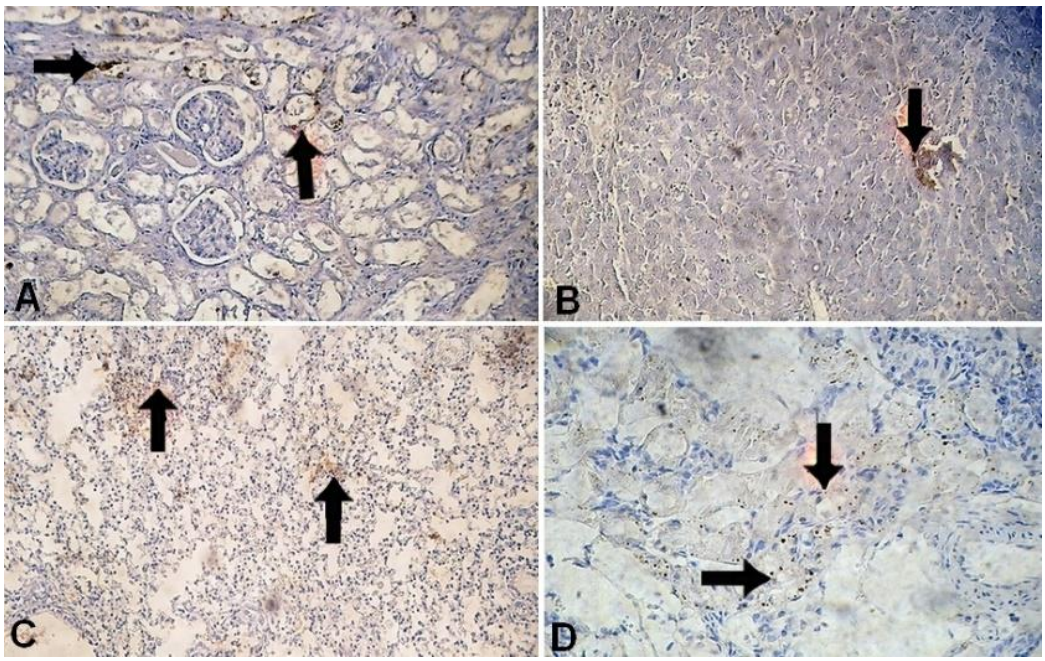
	Toplam Pozitif Örnek	Böbrek	Böbrek+Karaciğer	Böbrek+Akciğer	Böbrek+Akciğer+Karaciğer
IHC	160	125	15	13	7
Levaditi	108	108	-	-	-



Şekil 3. A. Böbrek tubulus lümenlerinde siyah renkte spiral bakteriler (ok), Levaditi, X400, B. Böbrek korteks tabakasında spiral bakteriler (oklar), Levaditi, X400, C. Böbrek korteksinde yaygın spiral bakteriler (oklar), Levaditi, X200, D. Böbrek tubulus lümeninde siyah renkte spiral bakteriler (ok), Levaditi, X200.

İmmunohistokimyasal Bulgular

Çalışmada İHK yöntemiyle 160 örnekte pozitif boyanma tespit edildi. Boyanmalar böbrek tubul epitellerinde intrasitoplazmik kahverengi granüller halinde ve bazen de bazal membrane yakın kısımlarda hücre zarına yapışık vaziyette görüldü (Şekil 4A). Pozitif boyanan 160 örneğin 125 tanesinde sadece böbrekte pozitiflik saptanırken, 15 tanesinde böbrek ve karaciğerde (Şekil 4B), 13 tanesinde böbrek ve akciğerde (Şekil 4C,D), 7 tanesinde de böbrek ile aynı zamanda karaciğerde hepatositlerin sitoplazmaları, akciğerde interalveolar ve interlobüler septum ile alveol lümeninde hücre içi pozitiflik saptandı. Olguların tümünde (160) böbreklerde pozitiflik vardı (Tablo 3).



Şekil 4. Anti-leptospira rabbit antiserumu ile; A. Tubul epitel hücrelerinin luminal yüzeylerinde kahverenkte granüler boyama (oklar), böbrek, IHC, X200, B. Hepatositlerde granüler boyama (ok), karaciğer, IHC, X200, C. İnteralveolar septumda kahverenkte granüler boyama (oklar), akciğer, IHC, X200, D. Alveollerde hücre içi granüler boyama (oklar), akciğer, IHC, X200.

TARTIŞMA

Leptospiroz, abort, ölü doğum, erken doğum, infertilite, septisemi, nefritis, hemoglobinüri, hepatitis, mastitis gibi semptomlarla karakterize zoonoz bakteriyel bir enfeksiyondur. Hayvanlarda infertilite, ölü doğum, abort, ölüm ve süt veriminde düşüşlere neden olduğu için hayvancılık ekonomisi açısından çok önemli bir hastalıktır (Bolin, 2005).

Amerika Birleşik Devletleri (ABD), İngiltere, Avustralya, Yeni Zellanda, eski Sovyetler Birliği ülkeleri, Avrupa ve Asya'daki birçok ülkeden leptospiroz bildirimi yapılmıştır. İran'da yapılan bir çalışmada %25-42 arasında *Leptospira spp.* seropozitifliği saptanmıştır (Hamali vd., 2012). Hastalığın prevalansına yönelik yapılan çalışmalarda Portekiz'de %15, Nijerya'da %14, Zimbabve %10, Çek Cumhuriyetinde %7 olarak bildirilmiştir (Hamali vd., 2012). Ayrıca Nikaragua, Brezilya, Hindistan, Güneydoğu Asya ve ABD'inde salgınlara neden olmuştur. Özellikle büyük su baskınlarından sonra birçok ülkede hastalık salgınlara neden olmuştur. El Nino kasırgasından sonra özellikle Orta ve Güney Amerika'da birçok vaka tespit edilmiştir (Levett, 2001).

Miller vd. (1991) ABD'nin 49 eyaleti ve Porto Rico'da yaptıkları çalışmada böbrek ve kan serumu örneklerini incelemişler ve kan serumu analizleri sonucunda %49 oranında pozitiflik saptamışlardır. Çalışma sonucunda böbreklerin %1,7'sinde leptospiraları izole etmişler, en sık görülen serovarların *L. hardjo (*%83), *L. pomona (*%12.5) ve *L. grippotyphosa (*%4.5) olduğunu bildirmişlerdir. Dünyada olduğu gibi ülkemizde de leptospirozun tanısı, baskın serovarları ve prevalansı üzerine birçok çalışma yapılmıştır. Ulaş ve Alver'in (1973) yaptıkları çalışmada Batı Anadolu ve Trakya'da Mikroskopik Aglütinasyon Testi (MAT) ile *L. grippotyphosa*'ya karşı %5,09, *L. sejreo'*ya karşı %3,4 ve *L. icterohaemorrhagiae'*ye karşı %0,104 pozitiflik bildirmişlerdir. Şahin vd. (2000), Kars ve Ardahan'da ELISA ve MAT testi ile çalışma yapmışlardır. Çalışma sonucunda MAT testi ile %33,63'ünde, ELISA testi ile %36,26'sında *L. hardjo* ve *L.*

grippotyphosa serovarlarına karşı antikor saptamışlardır. Ertaş ve ark. (2000), Doğu Anadolu Bölgesi'ndeki illerinde MAT ve ELISA ile çalışma yapmışlar ve çalışma sonucunda *L. hardjo, L. pomona, L. hebdomadis* ve *L. icterohaemorrhagiae*'ye karşı spesifik antikorlar tespit etmişlerdir. MAT testi ile %16 ve ELISA ile %23,3 oranında antikor saptamışlardır. Bu çalışma sonucunda ELISA spesifitesinin %84 ve sensitivitesinin %68 olduğunu bildirmişlerdir. Doğu Anadolu'daki bazı illerde hastalık sığırlarda %17,8 ve koyunlarda %2,8 oranında serolojik olarak saptanmıştır (Bulu vd., 1990). Erzurum yöresinde yapılan bir çalışmada (Özkan vd., 1993), hastalığın sığırlarda %16, koyunlarda % 0,04 oranında seyrettiği görülmüştür. Sunulan çalışmada 2013-2018 yılları arasında Marmara Bölgesi'nden Pendik Veteriner Kontrol Enstitüsüne gelen büyükbaş ve küçükbaş ruminant fötüsları İHK ve Levaditi yöntemleriyle incelendi. Çalışmada 1252 adet abort fötüstan İHK metodu ile 160 adetinde (%12,77) pozitiflik saptanırken, Levaditi boyama yöntemi ile 108 örnekte (%8,6) leptospira etkenleri gözlendi. İHK metodu ile yapılan incelemelerde, 750 adet koyun fötüsunun 76 adedinde (%10,13), 218 adet keçi fötusunun 26 adedinde (%11,92) ve 284 adet sığır fötusunun 58 adedinde (%20,42) leptospiroz pozitif olarak tespit edildi.

Elde edilen bulgular iller bazında incelendiğinde; Edirne (%17,94), Kırklareli (%13,29), Tekirdağ (%9,83), Kocaeli (%11,21), Yalova (%7,5), Sakarya (%6,77), Bursa (%9,16), Bilecik (%14,06), Balıkesir (%15,54), Çanakkale (%12,72), Düzce (%11,47) ve İstanbul (% 0) oranlarında pozitiflik bulunmuştur. Çalışma sonucunda elde edilen il bazındaki verilerden en yüksek pozitifliğin Edirne'de %17,94 oranında olduğu görülmüştür. Edirne'de pirinç tarlalarının yoğun olması ve buna bağlı olarak etkenin sulak arazileri sevmesi ve yüzey sularında kolayca üreyebilmesi pozitifliğin yüksek olmasını açıklamaktadır. İstanbul'da pozitiflik oranının % 0 olarak belirlenmesi, gelen örnek sayısının çok az olmasından (8 olgu) kaynaklandığını düşündürmüştür.

Dokulardan etken izolasyonunun yapılabildiği, fakat etkenin hızla ölmesi ve örneklerin taze olmaması izolasyon aşamasında güçlük çıkarmaktadır (Ellis, 1983; Maxie, 1993). Gümüşleme teknikleri ile etkenin iyi boyanabilmesi için dokunun tespit edilmeden önceki tazeliği önem arz etmektedir. Aksi durumlarda etkenin görünümlerinde değişimler olduğu belirtilmektedir (Falini ve Taylor, 1983; Russell ve Faine, 1984). Oluşan böyle güç durumlar karşısında immunperoksidaz metodu ile leptospiroz tanısını koymanın daha kolay (Ellis, 1983; Scanziani 1991), hassasiyetin %78 ve doğruluğunun %100 olduğu bildirilmiştir (Scanziani, 1991). Hathaway vd. (1982), MAT testini kullanmışlar ve pozitiflik saptadıkları örneklerden de izolasyon çalışması yapmışlardır. Aborte sığır fötüslarının böbrek, akciğer, karaciğer ve kan örneklerinden yaptıkları çalışma sonucunda, sadece aborte fötüs böbreklerinden leptospira izole etmişlerdir. Benzer şekilde sunulan çalışmada da Levaditi yöntemiyle sadece aborte fötüslerin böbreklerinden etken tespiti yapılmıştır.

Sağlam vd. (2008) abort fötuslarında, Yener ve Keleş (2001) ise leptospira şüpheli sığırların dokularına yapılan Levaditi boyamalarında etkenleri tespit edemediklerini fakat immunperoksidaz boyamada leptospira antijenlerini tespit ettiklerini bildirmişlerdir.

Abortusların tanısını koyabilmek açısından serolojik testlerin yeterli olamayacağını, bu çalışmaların immunperoksidaz gibi yöntemler ile desteklenmesi gerektiği (Kıran vd.,1997) belirtilmiştir. Leptospira etkenlerinin genelde böbrek tubuluslarının lumenlerine yerleştiği (Ellis, 1983; Scanziani, 1991) ve şiddetli nefritis tablosu oluşturduğu bildirilmektedir (Maxie, 1993). Bu çalışmada İHK yöntemiyle koyun fötuslarında hafif, orta, keçi fötuslarında orta ve güçlü derecede, sığır fötuslarında orta ve güçlü

derecede antijen tespiti yapılan diğer çalışmalarla (Szeredi ve Haake, 2006; Sağlam vd., 2008) uygunluk göstermektedir. Araştırıcılar (Sağlam vd., 2008, Szeredi ve Haake, 2006) abortuslarda leptospirozun İHK ve Levaditi yöntemleri ile tanıda başta böbrek olmak üzere karaciğer, akciğer, plasentada antijen ve etken tespit ettiklerini bildirmişlerdir. Sunulan çalışmada da İHK yöntemiyle böbrekte yoğun olmak üzere karaciğer ve akciğerde antijen pozitifliği tespit edilirken, Levaditi yöntemiyle sadece böbreklerde etkenlere rastlanmıştır.

Yapılan çalışmalarda, anti-leptospira rabbit antiserumu ile boyanma tarzının, dalga formunda ve kümeler şeklinde olduğu bildirilmiştir. Çalışmamızda da hem dalga formunda hem de yoğun antijen kümelenmeleri şeklinde boyanmalar diğer çalışmalarla (Sağlam vd., 2008; Szeredi ve Haake, 2006) uyumludur. Sunulan çalışmada İHK yöntemiyle yapılan boyamalarda leptospira antijenleri, böbrekte tubulus epitel hücreleri, karaciğerde hepatositlerin sitoplazmaları, akciğerde interalveolar ve interlobüler septum ile alveol lümeninde hücre içinde tespit edildi.

Sunulan çalışmada, teşhis amacıyla gönderilen aborte fötüsların çoğunda makroskobik olarak otolitik değişiklikler görülmüştür. Bu olgulardan hazırlanan preparatların incelemeleri sırasında da otolitik değişiklikler nedeniyle histopatolojik bulguların değerlendirilmesi organ bazında ve istenilen düzeyde yapılamamıştır. Bu nedenle İHK ve Levaditi yöntemleriyle negatif sonuçlar elde edilen olgulara ait histopatolojik bulguların değerlendirme ve yorumlarının da eksik veya yanlış olabileceği kanısına varılmıştır. İHK ve Levaditi yöntemleriyle pozitif sonuç elde edilen olgulara ait gözlenebilen mikroskobik değişiklikler dikkate alınarak histopatolojik bulgularla karşılaştırılması yapılabilmiştir.

SONUÇ

Sonuç olarak, ruminant abortuslarında leptospira enfeksiyonlarının izolasyon zorluğundan dolayı leptospirozun spesifik ve erken tanısında İHK yönteminin güvenli sonuçlar verdiği, ayrıca Levaditi metodunun da tanıda kullanılabileceği gösterilmiştir. Çalışmada elde edilen sonuçlar, Marmara Bölgesi'ndeki ruminant abortuslarında leptospira enfeksiyonlarının önemini ortaya koymuş, ülkemiz ve dünyada önemli bir zoonoz olan leptospiroz ile mücadelede aşılamanın, kemiricilerle mücadelenin ve hastalıkla ilgili olarak toplum farkındalığının arttırılmasının önemli olduğu kanaatine varılmıştır. Elde edilen sonuçlar ile hayvancılık sektöründe ve ülke ekonomisinde önemli ekonomik kayıplara neden olan leptospiroza bağlı abort olgularının önlenmesi ve gerekli tedbirlerin alınması için konunun önemine dikkat çekilmek istenmiştir.

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Assessment of calpastatin and insulin-like growth factor 1 genotypes in Tsigai sheep

Research Article

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ABSTRACT

The aim of the present study was to evaluate the genotypic distribution of calpastatin (*CAST*) and insulin-like growth factor 1 (*IGF1*) gene polymorphisms in the Tsigai sheep breed. Phenol-chloroform extraction procedures were applied to extract genomic DNA. A total of 56 sheep were genotyped by the PCR-RFLP method. Frequencies of the alleles/genotypes were calculated by the standard procedures. To evaluate the population-genetic properties, the Hardy–Weinberg Equilibrium (HWE) testing was performed. Moreover, genetic diversity was evaluated through the number of effective alleles (Ne), heterozygosity (He), polymorphism information content (PIC), the level of possible variability realization (*V*%), the fixation index (F_{IS}), and the Shannon-Weaver diversity index (*H´*) were estimated. Results revealed that the heterozygous genotype (0.64) frequency was remarkably higher than the homozygotes in the *CAST* locus. HWE testing showed a deviation ($P<0.05$) and the estimation of population genetic parameters indicated a moderate genetic variability in the *CAST* marker. Concerning *IGF1*, the Tsigai population was found to be monomorphic. In this context, all the animals were genotyped as the BB. The results provided by the present study may be useful in evaluating the genetic structure of the Tsigai sheep breed for which limited information is available.

Keywords: Tsigai breed, sheep, PCR-RFLP, *CAST*, *IGF1*

INTRODUCTION

Sheep breeding is one of the most significant sectors of livestock. It provides high-quality food and essential raw materials that are necessary for many branches of industry (Ilişiu et al., 2013; Yu et al., 2013; Sheriff and Alemayehu, 2018). In this context, many different sheep breeds have been developed worldwide concerning varying purposes. Tsigai sheep is a multi-purpose breed with a main focus on cheese production. This breed is one of the most important native sheep breeds in Central, South-Eastern, and Eastern Europe. They have white wool, brown, reddish, or white face, and legs. Tsigai is a medium-sized sheep that has an angular form, with a long and thin tail (Ilişiu et al., 2013). Although the homeland of this breed is Romania, it is imported and raised in different regions of Europe and Central Asia, such as Turkey.

Selection for low-heritability quantitative traits of economical importance is rather complicated and therefore difficult to benefit in breeding programs. Over the last decades, researchers in the field of livestock have been focused on the genotypic evaluation of these traits. Hereupon, the search for quantitative trait loci (QTL) and effective genetic markers significantly contribute to the variance of trait expression has been a primary interest for animal breeding and genetics.

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Various genetic markers have been known to be potentially associated with specific traits analyzed. Among them, calpastatin (*CAST*) is one of the important genes in livestock and it encodes the specific inhibitor of the calcium-dependent calpain protease family. Thus, it plays a fundamental role in muscle growth (Palmer et al., 1998; Schenkel et al., 2006; Gabor et al., 2009; Bayram et al., 2019). Ovine *CAST* gene is located on chromosome 5 and the length of its transcript is 2,701 bps (Ensembl, 2021). This gene has been reported to be a remarkable marker in selection aiming to improve meat quality and production (Casas et al., 2006; Schenkel et al., 2006; Zhou et al., 2007; Ardicli et al., 2017). Another important gene involves in physiological processes including reproduction and growth is insulin-like growth factor 1 (*IGF1*) which plays a pivotal role in mammalian fertility (Siadkowska et al., 2006; He et al., 2012; Bayram et al., 2019). It is thus a strong candidate gene for reproductive traits in livestock species. This gene has been mapped to chromosome 3 in sheep (Ensembl, 2021). The variation in the *IGF1* gene is associated with growth and reproductive traits in sheep (Curi et al., 2005; He et al., 2012; Ardicli et al., 2019).

The concept of breeding programs has been gradually improved by changing the main applications from phenotypical selection to genotypic selection by using molecular techniques. Genetic evaluation is needed to achieve desired sustainability and profitability and to implement an efficient livestock improvement. The knowledge of variation in the major genes in different breeds is one of the most indispensable constituents in genetic assessment. *CAST* and *IGF1* genes are significantly associated with growth and reproduction performance in livestock. These genes play fundamental roles in important traits of sheep as in other ruminants. In this study, the variation in the ovine *CAST* and *IGF1* genes has been investigated in the Tsigai breed.

MATERIAL and METHOD

Animals and sampling

In total 56 Tsigai sheep were investigated. All animals were raised in the Bursa Uludag University, Faculty of Veterinary Medicine Practice and Research Farm, located in the South Marmara region of Turkey (40° 14′ N and 28° 52′ E). The study was carried out in compliance with the ethical requirements and was approved by the local Ethics Committee for Animal Research (App. No: 2020-05/12). Blood samples were collected into 5mL K_3EDTA vacutainer tubes from the vena jugularis of each sheep.

DNA isolation

Genomic DNA was isolated from blood samples using a phenol/chloroform method (Green and Sambrook, 2012). The spectrophotometric quantification of 1 µl of each DNA sample was carried out through the NanoDrop 2000c (Thermo Scientific, Wilmington, DE, USA).

Genotyping

Genotyping of the single nucleotide polymorphisms (SNPs) in the *CAST* and *IGF1* genes was performed by PCR-RFLP. PCR conditions, primer sequences, and restriction enzymes are shown in Table 1. PCR amplification was performed in a 25 µL reaction containing ~50 ng of genomic DNA, 12.50 µL PCR master mix (OneTaq Quick-Load 2x MM with Standard Buffer, New England BioLabs Inc., Ipswich, Cat#M0486S, USA), 1 µL (0.50 µM) of each primer, and 8 µL of nuclease-free water (Thermo Scientific Inc, USA, Cat#R0581). The PCR was performed using the Palm Cycler GC1-96 (Corbett Research, Australia). Amplification products were checked on a 2% agarose gel (migration

for 50 min at 90 V) using 5 µL of PCR product and 2 µL of loading buffer. RFLP analysis was performed by incubating a mixture of 15 µL of PCR product, 7.50 µL of nuclease-free water (Thermo Scientific), 3 µL of 10x enzyme buffer, and 0.50 µL of *Bst*H2I (RGCGC/Y; New England Biolabs, Beverly, MA, USA) at 37˚C for 16 h. Gels were visualized using a 3% agarose gel, photographed, and analyzed using a gel imaging system (DNR-Minilumi, DNR Bio-Imaging Systems, Israel).

Table 1. Primer sequences (from 5' to 3'), PCR conditions, and restriction enzymes that were used for genotyping the polymorphisms in the current study

Locus	Amplicon (bp)	Primers (5' to 3')	PCR conditions	Restriction enzyme	Reference
CAST	622	F: 5' TGGGGCCCAATGACGCCATCGATG 3' R: 5' GGTGGAGCAGCACTTCTGATCACC 3'	95°C 5′ (94°C 1′, 60°C 1′, 72°C 2′) 30 cycles, 72°C 10′	*Msp*I	Palmer et al.[6]
IGF1	294	F: 5'TGAGGGGAGCCAATTACAAAGC3' R: 5'CCGGGCATGAAGACACACACAT3'	94°C 6′ (94 °C 30s, 55°C 30s, 72°C 30s) 30 cycles, 72°C 10′	*Bst*H2I*	He et al.[12]

CAST: calpastatin; *IGF1*: insulin-like growth factor 1; * isoschizomer of *Bsp*143II

Evaluation of genotypic data

The allelic/genotypic frequencies were estimated using standard procedures (Falconer et al., 1996). Deviation from Hardy–Weinberg equilibrium (HWE) was calculated for each locus by using the chi-square goodness-of-fit test. Population genetic parameters including heterozygosity (He), effective allele numbers (Ne), and the polymorphism information content (PIC) were calculated according to Nei and Roychoudhury (1974) and Botstein et al. (1980). The level of possible variability realization (*V*%) was estimated according to Crow et al. (1970) as follows:

$V\% = (1\text{-}E) / (1\text{-}1/N) \times 100$

where:

E= expected homozygosity

N= number of individuals in a population regarding a particular locus

The fixation index (F_{IS}) was estimated from the values of theoretical (H_{the}) and experimental (H_{exp}) heterozygosities using the following formula:

$$Fis = \frac{Hthe - Hexp}{Hthe}$$

The Shannon-Weaver diversity index (H') was calculated as follows:

$$H' = -\sum_{n=1}^{n} P_i^2 \ln P_i$$

where:

P_i is the proportion of each species/taxa/allele in the population, and ln is the natural logarithm (Ortiz-Burgos, 2016; Shannon and Weaver, 1949).

RESULTS

In the *CAST* gene, the reaction was performed to amplify partial regions of exon 1C and 1D and the intron between them. A 622 bp PCR product for the *CAST* gene (Figure 1) was digested with the *Msp*I restriction endonuclease and the reaction differentiated alleles M and N.

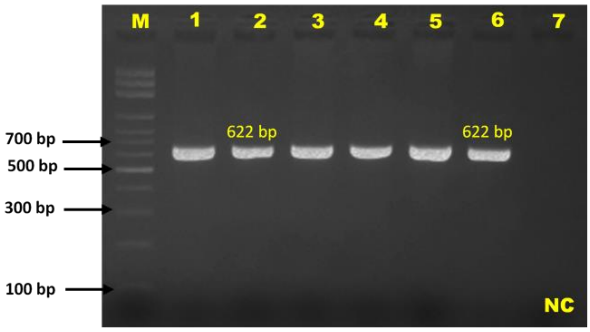

Figure 1. The electrophoresis pattern of PCR amplification (622 bp amplicon) for ovine *CAST* locus

(M: Marker, 100-1500bp; NC: Negative control; bp: Base pair)

The results indicated three genotypes (MM, MN, and NN) were detected in Tsigai sheep (Figure 2). The homozygous genotype MM (336 bp, 287 bp) was detected in 14 sheep while the NN genotype (622 bp) was observed in six sheep. The heterozygous genotype MN (622 bp, 336 bp, 287 bp) was detected in 36 sheep.

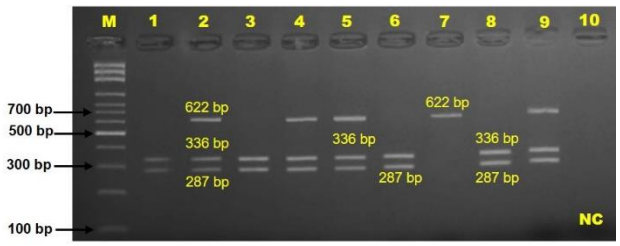

Figure 2. The electrophoresis pattern of restriction enzyme digestion of PCR product with *Msp*I for ovine *CAST* genotypes including NN and MN (M: Marker; NC: Negative control; bp: Base pair; Lines 1, 3, 6, and 8: MM; Line 7: NN; Lines 2, 4, 5, and 9: MN; Line 10: NC)

These results indicated a remarkable high frequency of heterozygous genotype carriers (64.29%). Notably, the NN genotype was very low (10.71%). The M allele frequency (0.57) was higher than the N allele frequency in the studied Tsigai population (Table 2). The genotypic distribution was not following the HWE (P<0.05). The calculation of population genetics indices revealed an admissible genetic variability regarding He (~0.49), Ne (~1.96), and PIC (~0.37). The F_{IS} and V% were found to be 0.2657 and 0.4990, respectively. The H' value indicated an acceptable diversity level (Table 3) in the Tsigai breed.

Table 2. Allele and genotype frequencies of *CAST* and *IGF1* in Tsigai sheep

Gene	Genotype Frequency*			Allele Frequency	
	MM	MN	NN	M	N
CAST	0.25 (14)	0.64 (36)	0.11 (6)	0.57	0.43
	AA	AB	BB	A	B
IGF1	0	0	1 (56)	0	1

*The number of animals per genotype is presented in parentheses

The amplification of the 5' regulatory region of the *IGF1* gene using the appropriate primers yielded a 294 bp amplicon. However, *Bst*H2I enzyme digestion (Figure 3) revealed that the B allele was fixed in the studied Tsigai sheep population because only the BB genotype was found (Table 2). Accordingly, the HWE and population genetic indexes for this locus were not estimated.

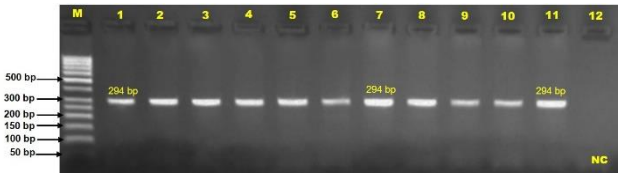

Figure 3. The electrophoresis pattern of the ovine *IGF1* locus. PCR revealed 294 bp amplicon; all samples remained undigested (*Bst*H2I restriction enzyme). Accordingly all samples were genotyped as the BB (M: Marker, 50-1000bp; NC: Negative control; Lines 1-11: BB; Line 12: NC; bp: Base pair)

Table 3. Population genetic indices and compatibility with the Hardy–Weinberg equilibrium of calpastatin gene in Tsigai sheep

Population genetics parameters	
Heterozygosity (He)	0.4902
Number of effective alleles (Ne)	1.9616
Polymorphism information content (PIC)	0.3701
Level of possible variability realization (V%)	0.4990
Fixation index (F_{IS})	0.2657
Shannon index (H')[a]	0.6833
The compatibility with the Hardy–Weinberg equilibrium	
χ^2	5.4687
P	0.0193*

[a]Also referred to as the Shannon-Weaver diversity index, Shannon's information index, or Shannon entropy
*P<0.05; not consistent with the Hardy–Weinberg equilibrium

DISCUSSION

The presence of sufficient genetic diversity is a key point to the maintenance and long-term survival of most species including sheep and goats. Regarding recent animal production, research efforts into QTLs are underway internationally (Khan et al., 2012). This evaluation should be performed based on comprehensive information regarding the structure of the populations and the sources of genetic variability within and among populations (Sheriff and Alemayehu, 2018). The genotypic variation in livestock has been created by the forces of both natural and artificial selection (Groeneveld et al., 2010). The adaptation of different sheep breeds to a broad range of agroecology provides adequate variability that provides opportunities to meet the increased future demands for food and offers flexibility to respond to changing markets and needs (Sheriff and Alemayehu, 2018; Wollny, 2003). These processes have resulted in a wide variety of sheep breeds that were improved according to the demands of particular countries. Sheep are species able to adapt to a broad range of environments. This has enabled them to be easily reared in regions very different from the geography they originated from. However, this may result in genetic structures different from the original breed concerning natural processes that cause variation or crossbreeding. Genetic evaluation is necessary for not only pure/crossbreeds but also the breeds raised in different countries. This study was performed to evaluate the genetic variability regarding the *CAST* and *IGF1* genes in Tsigai sheep raised in Turkey conditions. It is important to note that the level of genetic variability may be remarkably high between breeds. In fact, genetic differences are present between different populations of the same breed (Ardicli et al., 2019). Evaluation of genotypic distribution of the major genes in livestock is required for providing a basic genetic data set through different sheep breeds raised in Turkey. In this respect, *CAST* is one of the best-known and popular major genes in livestock breeding. This gene encodes calpastatin which is an endogenous inhibitor of the calpains and its activity is associated with decreased rates of muscle protein turnover. This negative correlation results in increased levels of skeletal muscle growth (Chung et al., 2012; Goll et al., 1998). *CAST* plays important role in the formation of muscles, postmortem mechanisms related to muscle-specific systems, and thus, meat tenderness (Gabor et al., 2009). Hence, it has been shown to be a pivotal gene in meat quality evaluation (Schenkel et al., 2006). Therewithal, an association of the *CAST* with growth and live weight gain in sheep has been reported by Khan et al. (2012). The authors have reported that individuals with the heterozygous genotype were found to have higher weight gain compared to homozygotes in the native Balkhi and Kajli sheep breeds. However, the MN genotype frequency was quite lower in both sheep breeds they studied (Khan et al., 2012). Indeed, several studies have indicated that the MM genotype is predominant in various sheep breeds worldwide (Bayram et al., 2019; Dincel et al., 2015; Gabor et al., 2009; Gorlov et al., 2016; Khan et al., 2012). Gabor et al. (2009) have reported that the MM has the highest genotype frequency in Tsigai sheep and Tsigai × Lacaune crossbreds. They also reported that the NN genotype was not present in purebred Tsigai sheep. However, in the present study, we observed a quite different genotypic distribution compared to previous studies mentioned above. In this context, the predominant genotype was the *CAST-Msp*I-MN heterozygotes (0.64), and moreover, we have observed six sheep with the NN genotype. In accordance with the present results, Yılmaz et al. (2014) and Balcioğlu et al. (2014) have reported that the frequency of heterozygous genotype was the highest in Sakız and Karya sheep, respectively. Differences in the levels of

genetic variation and the frequencies of alleles can be considered as common circumstances in genetic studies conducted on different breeds or different populations of the same breed. However, remarkable differences in genetic variability in genetically well-known breeds or the absence of some common alleles may raise questions about breed purity. Nevertheless, this needs further investigation. Concerning the studied sheep breed in this study, Tsigai, there is limited genetic information even about very popular genetic markers for livestock. Hence, inconsistent results may not be surprising for this sheep breed.

In this study, there was a deviation from HWE regarding the selected *CAST* locus (P<0.05). This disequilibrium is a result of the substructure of populations under an intense selection process. Population genetic parameters are valuable indicators that provide knowledge on the genetic variation of populations concerning a particular gene or genes (Ardicli et al., 2019). Among these parameters, He and Ne express the quality and suitability of the genetic markers in a particular population. In this study, He approaches 0.50 whereas Ne approaches 2.00 in the Tsigai sheep population. Concerning the PIC observed (~0.37), the studied *CAST* marker can be considered as moderately informative (0.25<PIC<0.50) based on the classification suggested by Botstein et al. (1980). On the other hand, the indices of heterozygosity-homozygosity balance including F_{IS} and *V*% reflect eventual heterozygosity and they display the degree to which heterozygosity decreases (Duifhuis-Rivera et al., 2014; Miluchová et al., 2013). In this study, *V*% and F_{IS} were found to be 0.50 and 0.26, respectively. The H' is one widely used index for comparing diversity in biological systems (Ortiz-Burgos, 2016). This index can provide information to describe variation at multiple levels of genetic organization from SNPs, through whole species or larger taxonomic units to ecosystems (Konopiński, 2020). Estimation in the present study revealed that the H' amount was ~0.68, which represents a high genetic variation for the exon 1C/1D of the ovine *CAST* gene in the Tsigai breed. The H' value in this study was remarkably high compared to a previous study conducted on Tsigai (H'=0.30) and Tsigai × Lacaune (H'=0.33) sheep (Gabor et al., 2009). It is important to note that the usefulness of a marker is directly related to its level of polymorphism, and thus, the *CAST Msp*I marker may be evaluated as an admissible genetic variation constituent. The limitation of the present study is the small sample size, yet it should be considered that few animals are belonging to this breed raised in Turkey.

IGF1 gene plays a crucial role in the regulation of growth, development, and reproduction (Bakhtiar et al., 2017). In this study, polymorphism located on the 5' regulatory region of the *IGF1* gene was evaluated in Tsigai sheep. It is important to note that, although of limited importance, there is no information on *IGF1* in this breed. The results revealed that the studied population was monomorphic for the *IGF1* locus. All of the examined animals were found to be BB genotype carriers. Similarly, Bayram et al. (2019) have reported that lambs of the Akkaraman breed were found to have the monomorphic BB genotype. On the contrary, He et al. (2012) showed that all three genotypes (AA, AB, and BB) were present in Small Tail Han sheep and Hu sheep breeds. Dorset sheep were monomorphic regarding the AA genotype. The AA and AB genotypes were present in the Texel breed. Notably, Dorset and Texel sheep were characterized by very high AA genotype frequencies (100 and 93.80%, respectively). Grochowska et al. (2017) have indicated that the A allele was predominant (91.60%) resulting in the higher frequency of the AA (83.30%) compared to heterozygotes in Coloured Polish Merino sheep (the BB genotype was absent) concerning

polymorphism in the 5' flanking region of the *IGF1* gene. Based on the results from previous studies, it may be plausible to interpret that the mentioned region of the ovine *IGF1* gene limitedly polymorphic. Nevertheless, the differences among sheep breeds with varying genetic backgrounds should not be overlooked.

CONCLUSION

This paper focused on the genetic variability of the ovine *CAST* and *IGF1* in Tsigai sheep. Heterozygous genotype was found to be predominant in the *CAST* locus. The frequency of the NN genotype was the lowest in the population, which consequently resulted in the lower frequency of the N allele than the M. Population-genetic analysis showed that the *CAST Msp*I marker is substantially informative for the Tsigai breed. Concerning the *IGF1* locus, the sheep population was found to have the monomorphic BB genotype. The present results may contribute to the current genetic information on the Tsigai sheep breed.

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Ethical approval: This study was approved by Local Ethical Committee of Uludag University with 2020-05/12.

Conflict of interest: The authors declare no conflict of interest.

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Melanistik sülün etlerinin bazı fiziko-kimyasal özelliklerinin araştırılması

Investigation of some physico-chemical properties of melanistic pheasant meats

ÖZET

Bu çalışmanın amacı farklı yetiştirme koşullarında tutulan melanistik halkalı sülünlerden elde edilen etlerin fiziko-kimyasal özelliklerini belirlemektir. Bu amaçla üç farklı grup oluşturularak sülünler yetiştirilmiştir. Birinci gruptaki sülünler açık kümeste serbest olarak gezebilecek şekilde sürü halinde, II. gruptaki sülünler yarı açık tel ızgaralı altlıklı bir kümeste sürü halinde serbest gezinmeli ve III. grupta bulunan sülünler ise kapalı bir kümesteki 3 katlı geleneksel yumurtacı tavuk kafeslerinde her bir kafeste tek sülün olacak şekilde bireysel olarak büyütülmüşlerdir. Sülün etlerinden alınan numunelerden protein değerleri I., II. ve III. gruplarda erkek ve dişi olarak sırasıyla; %19.25-17.08, %18.92-17.10 ve %17.14-16.87 olarak tespit edilmiş ve gruplar ve cinsiyetler arasında protein oranı bakımından önemli bir fark tespit edilmemiştir. Sülün etlerinin kül oranları I. ve III. gruplarda benzer, II. grupta ise farklı bulunmuştur ($p<0.05$). Kül oranı bakımından cinsiyetler ve numunelerin alındığı but ve göğüs bölgeleri benzer olarak tespit edilmiştir. But etlerinin pH değerlerinin göğüs etine göre önemli düzeyde yüksek ($p<0.05$) olduğu belirlenmiştir. Ancak pH değerleri bakımından gruplar ve cinsiyetler arasında önemli bir farklılık tespit edilmemiştir. *L** ve *b** renk parametreleri bakımından yetiştirme tipi ve cinsiyet grupları arasında önemli farklılık bulunmazken, göğüs eti değerlerinin but etlerine göre daha yüksek olduğu gözlemlenmektedir ($p<0.01$). *a** parametresi bakımından analizin alındığı lokasyon arasındaki farklılık ve lokasyon x cinsiyet interaksiyonu önemli bulunmuştur ($p<0.05$).

Anahtar Kelimeler: Et kalitesi, fiziko-kimyasal özellik, melanistik sülün, sülün eti

ABSTRACT

The aim of this study was to determine the physico-chemical properties of meat obtained from melanistic ringed pheasants raised in different housing conditions. For this purpose, three different groups were formed as the pheasants in the first group were raised in open fenced pens, in the second group the pheasants were raised in closed and semi-open coops, and the 3rd group was raised individually in 3-storey traditional layer hen cages. The protein ratios of male and female pheasants in the 1st, 2nd and 3rd groups were determined as 19.25%; 17.08%; 18.92% and 17.10%; 17.14%; 16.87 respectively. There were no significant differences between groups and genders in terms of protein ratio. Ash ratios of pheasant meats were similar in the 1st and 3rd groups, but the 2nd group was found different ($p<0.05$). In terms of ash ratio, the gender and the location (drumstick and breast regions) from which the samples were taken was found to be similar. It was determined that the pH values of drumsticks were significantly higher ($p<0.05$) compared to breast meat. However, no significant difference was found between the groups and genders in terms of pH values. While there was no significant difference between rearing type and gender in terms of *L** and *b** color parameters, it was observed that breast meat values were higher than drumstick ($p<0.01$). In terms of *a** parameter, the difference between the location where the analysis was taken and the location x gender interaction was found to be significant ($p<0.05$).

Keywords: Meat quality, physico-chemical properties, melanistic pheasant, pheasant meat

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Research Article

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- http://dergipark.org.tr/vetbio

GİRİŞ

Küresel gıda tüketim verileri, et ve et ürünlerinin insan beslenmesinin temel bileşenlerinden biri olduğunu ve olmaya devam edeceğini açıkça göstermektedir. Diğer gıda gruplarına benzer şekilde, çeşitli et türlerinin popülaritesi hem yerel hem de küresel olarak değişkenlik göstermektedir. Kanatlı, domuz ve sığır eti en fazla tüketilen gıdalar olmakla birlikte (OECD, 2019), diğer hayvanlardan elde edilen etler (kuzu/oğlak eti, at eti, tavşan eti, keçi eti ve av hayvanı etleri gibi) çok daha az tüketilmektedir. Bununla birlikte, tüketicilerin eğitim ve beslenme bilinci seviyelerinin artması, farklı et türlerine olan arzın artması ve ayrıca gıda kalitesi ve beslenmenin sağlık üzerindeki etkisi hakkında daha fazla bilimsel verinin ortaya konulması nedeniyle alternatif etlerin popülaritesi artmaktadır. Özellikle batı ülkelerinde tüketiciler, kolorektal kanser ve kardiyovasküler hastalıklarla ilişkilendirildiğinden dolayı kırmızı et tüketimi azalma eğilimi göstermektedir (Lopez-Pedrouso vd., 2019). Bu riskleri azaltmak için daha düşük yağ ve daha yüksek protein düzeyine sahip olmalarından dolayı av hayvanı etleri kırmızı ete alternatif olarak düşünülmektedir (Neethling vd., 2016). Kanatlı av hayvanı yetiştiriciliği, hastalıklara daha yüksek direnç göstermeleri, daha erken cinsel olgunluğa erişmeleri ve farklı yetiştiricilik koşullarına daha iyi uyum sağlamaları gibi çeşitli avantajları nedeniyle ekonomik olarak uygun ve ticari olarak uygulanabilirdir (Kilonzo-Nthenge vd., 2008).

Halkalı sülün yetiştiriciliği birçok ülkede olduğu gibi Türkiye'de de önem kazanmaya başlamıştır. En çok Amerika Birleşik Devletleri (ABD), İngiltere, Fransa ve İtalya'da yetiştirilen halkalı sülünler, ABD ve İngiltere gibi ülkelerde de av maksadıyla entansif olarak yetiştirilirler (Kırıkçı, 2012). Türkiye'de halkalı sülünler genelde Tarım ve Orman Bakanlığı'na bağlı üretim istasyonlarında tabii hayatı zenginleştirmek maksadıyla üretilirler. Fakat son zamanlarda ülkemizde, yetiştiriciliği yaygın yapılan ülkelerdeki gibi en önemli amacı avlak sektörüne materyal kazandırılması haline gelmiştir. Zaten, ülkemizin coğrafi ve iklim şartları entansif sülün yetiştiriciliği için çok uygundur ve sülün yetiştiriciliğinin ülkemizde de et üretim amaçlı olarak yapılabileceği düşünülmektedir (Çetin ve Kırıkçı, 2000; Kırıkçı, 2012).

Melanistik sülünler, halkalı sülünler arasından çıkan bir renk varyetesidir. Bu sülünlerin kendi aralarında yetiştirilmesi ile sürekli melanistik esmer renkte sülünler elde edilir. Halkalı sülünlerin ana vatanı olarak Türkiye'nin Karadeniz ve Marmara Bölgelerinin kıyıya yakın bölgelerinin içinde olduğu büyük bir coğrafyadan bahsedilmektedir. Anavatanlarının Türkiye ile Kafkas bölgelerini de içini alan bölgeler olan sülünlere Kafkas sülünü de denmektedir. Tarım ve Orman Bakanlığı'nın İstanbul Polonezköy ve Samsun Gelemen'de kurduğu tesislerde bu sülünler üretilmekte ve doğaya salınarak, yaşama bölgelerinde popülasyonlarını artırma çalışmaları yapılmaktadır. Bunun dışında halkalı sülünler Türkiye'de hobi ve özel avlaklara av materyali sağlamak amacıyla yapılmaktadır (Çetin ve Kırıkçı, 2000).

Araştırmalar, sülün etinin yüksek besin değerine ve arzu edilen duyusal özelliklere sahip olduğunu göstermektedir. Bu nitelikler; cinsiyet (Łukasiewicz vd., 2011; Kotowicz vd., 2012), yaş (Jakešová vd., 2014; Kokoszyński vd., 2014a), beslenme (Łukasiewicz vd., 2011; Kokoszyński vd., 2014b; Kokoszyński vd., 2018) ve karkas kesim şekli (Łukasiewicz vd., 2011; Quaresma vd., 2016) gibi çeşitli faktörler

tarafından şekillendirilmektedir (Daszkiwicz ve Janiszewski, 2020).

Bu çalışmada, farklı barındırma şartlarında (açık serbest gezinmeli, yarı açık-kafeslerde serbest gezinmeli ve kapalı kümeste bireysel kafeslerde) büyütülen melanistik halkalı sülünlerden elde edilen etlerin bazı fiziko-kimyasal özelliklerini belirlemek amaçlanmıştır.

MATERYAL VE METHOD

Araştırmanın materyalini Konya Bahri Dağdaş Uluslararası Tarımsal Araştırmalar Enstitüsünde yetiştirilen melanistik halkalı sülünlerden elde edilmiş olan sülün civcivleri oluşturmuştur. Araştırmada aynı tarihte çıkan sülün civcivleri 4 hafta ana makinelerinde birlikte büyütülmüşler ve 4 haftanın sonunda her bir grup için 30 adet sülün civcivi seçilmiştir. Gruplar için seçilen sülün civcivlerinin eşit sayıda cinsiyet içermesine (15 dişi, 15 erkek) dikkat edilmiştir.

Birinci gruptaki sülünler açık kümeste serbest olarak gezebilecek şekilde sürü halinde büyütülmüşlerdir. Bu amaçla I. grupta yer alan sülünler açık bir alanda 10 x 10 x 2.5 ebatlarında, yanları ve üzeri 2.5 cm^2 gözenekli çitlerle çevrilmiş açık bir kümeste (volyer) yetiştirilmiştir. II. gruptaki sülünler yarı açık tel ızgaralı altlıklı bir kümeste sürü halinde serbest gezinmeli olarak büyütülmüşlerdir. Kümes 5 x 4 x 2 m kapalı ve 5 x 4 x 2 m açık alanı ve 1.2 cm^2 gözenekli altlığa sahiptir. III. grupta bulunan sülünler ise kapalı bir kümesteki 3 katlı geleneksel yumurtacı tavuk kafeslerinde (45 × 30 × 35 cm), her bir kafeste 1 sülün olacak şekilde bireysel olarak büyütülmüşlerdir.

Rutubet Tayini

Sülün etlerinden numuneler göğüs ve butlardan 5 g'lık numuneler halinde deri ihtiva etmeyen etlerden alınmıştır. Rutubet analizleri AND MX-50 nem tayin cihazı kullanılarak yapılmıştır. Cihazın kefesine, küçük parçalar haline getirilen numuneden 5 g tartılıp, cihazın sıcaklığı 105 °C'ye ayarlandıktan sonra, ağırlık ibresi sabit kalıncaya kadar kurutulmuş ve göstergeden rutubet miktarı yüzde olarak okunmuştur (Telli vd., 2020).

Protein Tayini

Sülün göğüs eti numunelerindeki protein miktarları makro Kjeldahl cihazında belirlenmiştir (AOAC, 1984).

Kül Tayini

Sülün etlerindeki kül miktarı, Türk Standartları Enstitüsü tarafından oluşturulan TS 1746 standardına göre ölçülmüştür (TSE, 2001).

pH Analizi

Sülün etlerinden alınan numunelerin pH değerleri, dijital bir pH metre (InoLab pH 720 model, WTW, GmbH, Germany) ile belirlenmiştir.

Renk Analizi

Sülün etlerinin renk (*L**- parlaklık, *a**- kırmızılık ve *b**- sarılık) değerleri, Minolta marka renk cihazı (Minolta Camera Company, Keynes, UK) kullanılarak belirlenmiştir (Nute vd., 2007).

İstatistik Analiz

Kimyasal değerler ile ilgili verilerin analizinde GLM Univariate prosedürü uygulanmıştır. Oluşturulan modelde yetiştirme tipi, cinsiyet ve numunelerin alındığı lokasyon sabit faktörler olarak belirlenmiştir. Gruplar arası farklılıkların anlamlılık düzeylerini karşılaştırmada Bonferroni düzeltme testinden yararlanılmıştır. İstatistik analizler SPSS 25 Paket programı kullanılarak yapılmıştır (SPSS Inc, 2017).

BULGULAR

Sülün göğüs etlerinin protein oranları Tablo 1'de verilmiştir. Gruplar arasında protein düzeyleri bakımından istatistiki açıdan önemli bir farklılık bulunmamıştır ($p>0.05$).

Sülün etlerinin kuru madde, kül, ph ve renk değerleri Tablo 2'de sunulmuştur. Kül oranları

bakımından cinsiyetler ve numunelerin alındığı lokasyonlar arasında farklılık tespit edilmemiştir. Kapalı kafeslerde bireysel olarak yetiştirilen III. gruptaki sülünlerin but ve göğüs eti kül değerlerinin, II. gruptaki sülünlere göre daha yüksek olduğu görülmektedir ($p<0.05$). Volyerde toprak zeminde yetiştirilen sülünlerin (I. grup) kül değerlerinin diğer iki yetiştirme tipinin sahip olduğu değerlere benzer olduğu tespit edilmiştir.

But etinin pH değerinin göğüs etine göre daha yüksek olduğu görülmektedir ($p<0.05$). Cinsiyet ve yetiştirme grupları arasındaki pH değerleri bakımından önemli farklılık tespit edilmemiştir.

Tablo 1. Sülün göğüs etlerinin protein değerleri

Özellik	Grup I		Grup II		Grup III		SEM	p
	Erkek	**Dişi**	**Erkek**	**Dişi**	**Erkek**	**Dişi**		
Protein (%)	19.25	17.08	18.92	17.10	17.14	16.87	0.40	-

*L** ve *b** renk parametreleri bakımından yetiştirme tipi ve cinsiyet grupları arasında önemli farklılık bulunmazken göğüsten alınan örneklerin değerlerinin buttan alınanlara göre daha yüksek olduğu gözlemlenmiştir ($p<0.01$). But etinden elde edilen sonuçlara bakıldığında *L** değeri ortalamaları 42,16-50,23 iken göğüs etinde ise 52,42-58,28 arasında olduğu tespit edilmiştir ($p<0.001$). *a** parametresi bakımından analizin alındığı lokasyon arasındaki farklılık ve lokasyon x cinsiyet interaksiyonu önemli düzeyde bulunmuştur ($p<0.05$). Sadece erkeklerin but eti örneklerinin göğüs eti örneklerine göre önemli düzeyde daha yüksek olduğu görülmektedir. Yetiştirme tipi grupları arasında herhangi bir önemli farklılık gözlemlenmemiştir. Göğüsten alınan örneklerin % kuru madde değerleri, butlardan alınan örneklere göre daha yüksek bulunmuştur ($p<0.001$). Yetiştirme tipi ve cinsiyet bakımından % kuru madde değerleri arasında istatistiki açıdan önemli bir farklılık tespit edilememiştir. Lokasyon x Yetiştirme tipi arasında interaksiyon olduğu gözlenmiştir ($p<0.05$). Sülün etlerinin kuru madde, kül, ph ve renk değerleri Tablo 2'de verilmiştir.

Tablo 2. Sülün etlerinin kuru madde, kül, ph ve renk değerleri

	Grup I				Grup II				Grup III					
	Erkek		Dişi		Erkek		Dişi		Erkek		Dişi			
	But	**Göğüs**	**But**	**Göğüs**	**But**	**Göğüs**	**But**	**Göğüs**	**But**	**Göğüs**	**But**	**Göğüs**	**p**	**SEM**
KM[1] (%)	25.70^{a}	26.87^{b}	24.41^{a}	26.78^{b}	23.91	26.69^{b}	24.84^{a}	26.70^{b}	24.9	27.91^{b}	23.69^{b}	27.85^{b}	***	0.26
Kül (%)	1.13^{ab}	1.08^{ab}	1.16^{ab}	1.08^{ab}	1.15^{b}	1.12^{b}	1.04^{b}	1.08^{b}	1.22^{a}	1.17^{a}	1.20^{a}	1.14^{a}	*	0.01
pH	6.16^{a}	5.77^{b}	6.28^{a}	5.75^{b}	6.16^{a}	5.81^{b}	6.28^{a}	5.63^{b}	6.34^{a}	5.97^{b}	6.43^{a}	5.83^{b}	*	0.05
Renk														
Parlaklık (*L)**	42.16^{b}	52.42^{a}	46.66^{b}	54.75^{a}	50.23	55.56^{a}	44.07^{b}	56.63^{a}	48.5	55.06^{a}	45.70^{b}	58.28^{a}	***	1.17
Kırmızılık[2] (*a)**	9.48^{a}	8.31^{b}	7.98^{ab}	8.40^{ab}	10.25	7.90^{b}	8.72^{ab}	9.55^{ab}	10.3	6.85^{b}	8.26^{ab}	7.08^{ab}	*	0.28
Sarılık (*b)**	1.77^{b}	3.76^{a}	0.85^{b}	1.90^{a}	1.34^{b}	3.22^{a}	0.83^{b}	5.40^{a}	3.43^{b}	4.76^{a}	0.63^{b}	3.79^{a}	**	0.41

a.b; Aynı satırda farklı harf taşıyan değerler arasındaki fark önemli bulunmuştur ($p<0.05$)
*; $p<0.05$, **; $p<0.01$, ***; $p<0.0001$, SEM: Ortalamaların standart hatası
[1]Yetiştirme tipi x Lokasyon interaksiyonu önemli bulunmuştur ($p<0.05$)
[2]Yetiştirme tipi x Cinsiyet interaksiyonu önemli bulunmuştur ($p<0.05$)

TARTIŞMA

Genel olarak sülün eti, ideal duyusal niteliklere sahip, lezzetli av materyali olarak kabul edilmektedir. Sülünlerin en önemli ve en çok beğenilen ticari bölümleri but ve göğüs etidir. Ticari olarak satışa sunulan kısımların su içeriği özellikle sululuk ve yumuşaklık gibi tekstür ile ilgili özellikler bakımından et kalitesi üzerinde

büyük önem taşımaktadır (Lopez-Pedrouso vd., 2019). Yapılan araştırmada göğüsten alınan örneklerin % kuru madde değerleri buttan alınan örneklere göre daha yüksek bulunmuştur ($p<0.001$). Yetiştirme tipi ve cinsiyet bakımından % kuru madde değerleri arasındaki farklılık önemli değildir. Lokasyon x Yetiştirme tipi arasında interaksiyon olduğu gözlemlenmiştir ($p<0.05$). Protein değerleri bakımından gruplar arasında önemli bir farklılık bulunmamıştır. Daszkiewicz ve Janiszewski (2020) yaptıkları çalışmada 25 haftalık 18 erkek ve 18 dişi sülün etlerinin kalitesini araştırmışlardır. Araştırmacılar erkek ve dişi sülün göğüs etlerinde kuru madde ve protein düzeylerini sırası ile %27,26 – %27,81 ve %25,13-%25,43 olarak bildirmişlerdir. Kuru madde düzeylerinin mevcut araştırma ile paralellik gösterdiği görülürken, protein düzeyi bu araştırmadan daha yüksek olarak tespit edilmiştir. Protein düzeyinde görülen bu farklılığın hayvanların tükettiği rasyon içeriği, kesim yaşı ve genotipten kaynaklandığı düşünülmektedir. Grela vd. (2020) tarafından yapılan çalışmada 16 haftalık yaşta kesilen halkalı sülünlerin karkas özellikleri araştırılmıştır. Kuru madde düzeyleri mevcut araştırma ile paralellik gösterirken kül ve protein düzeyleri ise mevcut sonuçlardan daha yüksek bulunmuştur. Bu duruma da hayvanların beslenme şekilleri, rasyon içerikleri ve genotiplerinin farklı olmasının neden olabileceği düşünülmektedir. Flis vd. (2020) tarafından yapılan araştırmada 38 haftalık çiftlik sülünleri ve yabani hayattan avlanan sülünlerin karkas özellikleri karşılaştırılmıştır. Her iki grupta da kuru madde ve kül düzeyleri mevcut araştırma ile benzer olarak tespit edilmiştir. Ancak protein düzeyleri mevcut araştırmadan daha yüksek bildirilmiştir. % kuru madde değerleri Hofbauer vd. (2010) tarafından yapılan çalışmada elde edilen veriler ile kıyaslandığında ise tüm gruplarda değerler daha düşük bulunmuştur. Bu durum temel olarak hayvanların beslenme şekilleri (rasyon bileşimi) ve etlerin protein düzeylerinin farklı olmasından kaynaklanmaktadır (Lopez-Pedrouso vd., 2019).

pH'nın kanatlı etlerinin rengi, su kaybı ve raf ömrü üzerinde önemli etkileri bulunmaktadır. Depolama süresince pH'nın yükselmesi azotlu bileşiklerin ayrışması ve amonyak türevleri gibi diğer bileşiklerin oluştuğunu ve et kalitesinin bozulduğunu göstermektedir. Genellikle göğüs etinin daha düşük glikojen düzeyine sahip olması nedeniyle but etlerine göre daha düşük pH değerine sahiptir (Lopez-Pedrouso vd., 2019). Franco ve Lorenzo (2013) tarafından yapılan bir çalışmada göğüs etinin ortalama pH değerinin sülünlerde 5,69; Daszkiewicz ve Janiszewski (2020) ise erkek ve dişilerin göğüs etlerinde 5.70 ve 5.67 olarak bildirmişlerdir. Bu değerlerin mevcut araştırmada elde edilen sonuçlardan daha düşük olmasının sebebinin kesim ile ölçümün yapıldığı zaman arasındaki farklılıktan kaynaklandığı düşünülmektedir. Grela vd. (2020) tarafından yapılan bir çalışmada 16 haftalık yaşta kesilen sülünlerin but ve göğüs pH değerleri dişi ve erkek sülünlerde sırası ile 5.52–5.56 ve 5.56–5.56 olarak bildirilmiştir. Bu değerlerin mevcut araştırmada tespit edilenden daha düşük olduğu görülmektedir. Bu durum yine kesim ile ölçüm zamanı arasındaki farklılıktan kaynaklanmaktadır. Kokoszyński vd. (2012) tarafından yapılan çalışmada Mongolian x Versicolor melezi ve geleneksel sülünlerin karkas özellikleri karşılaştırılmıştır. Hayvanların 16 haftalık yaşta kesildiği ve analize alındığı ifade edilmiştir. Dişi ve erkeklerin but etlerinin pH düzeylerinin mevcut araştırmada daha düşük, göğüs etlerinin ise benzer düzeylerde olduğu görülmektedir. But etlerindeki bu farklılığın ölçüm derinliğinden kaynaklandığı düşünülmektedir. Hofbauer vd. (2010) tarafından yapılan çalışmada 29 adet doğadan avlanan erkek sülün ile 32 adet yetiştirilen dişi sülünün et kaliteleri

karşılaştırılmıştır. 24 saatlik dinlendirmenin ardından avlanan ve yetiştirilip kesimi yapılan hayvanların göğüs ve but etlerinin pH değerleri ortalama 5,66 – 5,55 ve 6,03 – 5,93 olarak bildirilmiştir. Hofbauer vd. (2010)'in sonuçlarıyla karşılaştırıldığında mevcut araştırmada bu değerler yüksek bulunsa da bu duruma pH ölçümlerinin kesimden hemen sonra gerçekleştirilmesinin neden olabileceği düşünülmektedir.

Et ürünlerinin renk parametreleri, tüketiciler tarafından etin kabulünü ve satın almayı etkileyen tazelik ile ilişkilendirildiğinden, önemli bir kalite kriteri olarak kabul edilmektedir. Diğer türlere ait etlerde olduğu gibi sülün etinin rengi de miyoglobin konsantrasyonu, pigmentlerin kimyasal durumu / oksidasyon düzeyi (Kannan vd., 2001), kas içi yağ seviyesi (Brewer vd., 2001), mikrobiyal bozulma (Stivarius vd., 2002) ve su tutma kapasitesi (Choe vd., 2009) gibi çeşitli faktörlerden etkilenmektedir. Daszkiewicz ve Janiszewski (2020) tarafından elde edilen renk değerleri karşılaştırıldığında L^* değerinin mevcut araştırmadan düşük, a^* değerlerinin volyer ve yarı açık besleme yapılanlardan düşük, bireysel beslenen gruptan yüksek olduğu, b^* değerlerinin ise mevcut araştırmadan daha yüksek olduğu görülmektedir. Renk değerlerinde meydana gelen bu farklılıkların kesim yaşı ve karkasların soğuk şartlarda muhafazaya alınıp alınmaması ile ilişkili olabileceği düşünülmektedir. But etlerinin kimyasal analiz sonuçları arasındaki ilişki yukarıda bahsedilen sonuçlar ile paralellik göstermektedir. Renk analizi sonuçlarında ise L^* değerleri mevcut araştırmada daha yüksek, a^* ve b^* değerleri ise daha düşük bulunmuştur. Bu durumun yine ölçümlerin yapılma zamanı ve hayvanların kesim yaşları ile ilgili olduğu düşünülmektedir. Kokoszyński vd. (2012) tarafından yapılan araştırmada göğüs etinin L^* değerlerinin mevcut araştırma ile benzer düzeylerde olduğu ancak a^* ve b^* değerlerinin daha düşük bulunduğu görülmektedir. Bu farklılığın kesim yaşı ve ırk farklılığından kaynaklandığı düşünülmektedir. Hofbauer vd. (2010) tarafından yapılan çalışmada göğüs etlerinin L^* değerleri mevcut çalışma ile benzer düzeylerde bulunmuştur. Ancak a^* değeri mevcut araştırmada daha yüksek bulunurken, b^* değeri daha düşük bulunmuştur. Kırmızılık ve sarılık değerlerinde meydana gelen bu farklılığın kesim yaşı, genetik grup ve yetiştirme tarzı gibi etkenlere bağlı olduğu düşünülmektedir (Fernye vd., 2017).

SONUÇ

Artan dünya nüfusunun ihtiyaçlarını karşılamak için önemli bir besin kaynağı olarak kabul edildiğinden, dünya genelinde et tüketimi artmaktadır. Bununla birlikte kırmızı et ile ilişkili sağlık sorunları tüketicileri farklı tür hayvan etlerine yöneltmektedir. Bu araştırmada üç farklı yetiştirme koşullarında büyütülen melanistik sülünlerden elde edilen etlerin fiziko-kimyasal özellikleri tespit edilmiştir. Gruplar ve cinsiyetler arasında but ve göğüs etlerinin kuru madde, protein, kül, pH ve renk değerleri bakımından farklılık bulunup bulunmadığı tespit edilmiştir. Yapılan literatür taramasında sülün yetiştiriciliği ve özellikle eti üzerine yapılan çalışmaların sınırlı olduğu görülmektedir. Sülün yetiştiriciliği bakımından önemli bir potansiyele sahip olan ülkemizde yapılacak ileri çalışmalar ile bu hayvanların yetiştiriciliğine katkı sağlanacağı ve bu hayvanlardan elde edilecek etlerin tüketiciler tarafından kabul edilebileceği düşünülmektedir.

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The analysis of the effects of propolis products as food supplements on the viability of baby hamster kidney (BHK-21) and murine macrophage (RAW 264.7) cells by spectrophotometric MTT assay

Research Article

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ABSTRACT

Propolis is beeswax with rich bioactive compound content. On the market, there are many propolis products as food supplements for the consumers. This study aimed to investigate the effect of food supplement products based on propolis at several concentrations on the viability of baby hamster kidney cells and murine macrophage cell lines (BHK-21 and RAW 264.7). For this purpose, both cell lines were treated with the two-fold serial dilutions (from 20 to 2-10) of each six propolis products (P1, P2, P3, P4, P5 and P6) after reaching monolayer cell in 96-well microplates. The viability and inhibition of cells were spectrophotometrically determined by MTT assay after 24 h. For BHK-21, the CC50s of P1, P2, P3, P4, P5 and P6 were calculated as 0.003, 0.178, 0,082, 0.451, 0.278 and 0.384 %, respectively. For RAW 264.7, the CC50 of P1, P2, P3, P4, P5 and P6 were calculated as 0.260, 0.218, 0.115, 0.257, 0.207 and 0.265%, respectively. The CC50 value was higher for RAW 264.7 cells than for BHK-21 cells. So, the low cytotoxic effect was determined in RAW 264.7 cells. Propolis products containing some additives (aroma, flavoring) had lower the CC50 and the lower viability of BHK-21 cells. So, Additives in the propolis food supplement might be an effective factor on cell viability as much as dilution factor and propolis content.

Keywords: BHK-21, Cytotoxicity, Food Supplement, Propolis, RAW 264.7

INTRODUCTION

Propolis is a beeswax containing natural ingredients, which bees produce from poplar and coniferous, clusia of flowers or tree and the hive cells (Gojmerac 1980; Valcic et al, 1999). Its bioactive compounds are generally flavonoids, ferulic acids, and terpenoids which come from resin, vegetable balsam, wax, essential aromatic oils, salivary secretions and pollen (Burdock, 1998; Yaghoubi et al., 2007; Bogdanov, 2014). But its composition varies related to the plant species, climate, season, harvest time, and geographic area (Markham et al. 1996; Sforcin et al. 2000). According to its chemical composition, propolis has many biological effects on anti-inflammatory and cellular immunity, wound healing and antioxidant metabolisms. It was shown that it has antimicrobial effects and also suggested that it might be used against COVID-19 (Burdock, 1998; Al-Shaher et al., 2004; Bedier, 2016; Martini and Mahendra, 2019; Berretta et al., 2020)

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Recently, there are many propolis product as food supplement for the consumers. In addition to propolis, these products contain various ingredients such as honey, royal jelly and plant flavoring. The bioactive compounds and concentrations in them are varied related to the solvents and their form (Moussa and Temirek, 2018; Berretta et al., 2020).

There are several test methods to determine the medical using safety of food or feed supplements before commercial production. Cytotoxicity assay is one of the most applied methods for establishing their safety in preclinical studies. Especially for the cytotoxicity methods, cell lines originated from mammalians such as kidney (baby hamster kidney, BHK-21) and blood (murine macrophage, RAW 264.7) were mostly preferred to understand the etiology of the diseases and to detect the levels of cellular toxicity in ethnobotanical and apitherapy studies. (Moussa and Temirek, 2018; Berretta et al., 2020). In this study, the aim was to investigate the effect of food supplement products based on propolis at several concentrations on the viability of two different cell lines (BHK-21 and RAW 264.7) by the spectrophotometric assay for assessing cell metabolic activity (tetrazolium-based colorimetric assay, MTT assay).

MATERIAL and METHOD

Materials

Trypan blue solution (0.4%, sterile-filtered, Sigma-Aldrich), DMEM (500ml, Dulbecco's Modified Eagle Medium (w/o L-glutamine, w/o sodium pyruvate)), L-alanyl-L-glutamine (200 mM), penicillin (10,000 units ml^{-1})-streptomycin (10 mg ml^{-1})-amphotericin B (0.025 mg ml^{-1}) solution, fetal bovine serum (FBS, European grade), trypsin-EDTA solution (w/o phenol red) and Dulbecco's Phosphate Buffered Saline (PBS w/o calcium magnesium) were purchased (Biological Industries, USA). For cell culture, the 75 cm2-flasks, (EasYFlask, Thermo Scientific) and 96-well microplates (CellStar, Greiner Bio-One, Germany), 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2Htetrazolium bromide (MTT, research-grade, Serva, Germany) was commercially supplied. The syringe filters (sterile, 0.22 µm, Merck Millipore, Germany), Sterile centrifuge tubes (ISOLAB, Germany) were also purchased.

Propolis samples

Six different propolis products were purchased from retail-markets and pharmacies in Istanbul, Turkey. All products were solved in water by their manufacturers and contained propolis at several concentrations. The ingredients of propolis products were shown in Table 1 and they were coded as P1, P2, P3, P4, P5 and P6. They were kept at 4 °C or room temperature in terms of manufacturers' recommendations for further analysis.

Cell culture

Baby hamster kidney cells (BHK-21) and murine macrophage cells (RAW 264.7) were from American Type Culture Collection (ATCC), Manassas, VA, USA). Fetal bovine serum was inactivated by heating up in a water bath at 56 ◦C for 30 minutes before use. All medium and solutions were heated to 37 °C before the process of cell cultivation. Both cells were cultured in DMEM supplemented with fetal bovine serum (10%), L-alanyl-L-glutamine and 1% penicillin-streptomycin at 37 °C. They were maintained in 75 cm^2 cell culture flasks in the incubator with the condition of 37 °C and 5% CO2 for 24 h to confluence. After the incubation of 24 h, all waste medium were discarded and the

monolayer cells were disaggregated with trypsin-EDTA in the incubator for 3 min. The suspension was centrifuged in a refrigerated centrifuge at 1200 rpm/15 min. The supernatant was discarded and the pellet was suspended in a fresh medium. Then, viable and dead cells were counted by the method of trypan blue (0.4%) staining with a haemocytometer. For the preparation of 96-well microplates, 100 µl of the stock viable cell suspension (3 x 10^5 cell ml^{-1}) was seeded in each well (3 x 10^4 cell) and kept in the incubator for 24 h to confluence at least 90%.

Propolis treatment on cell culture

All propolis solutions (P1, P2, P3, P4, P5 and P6) were 2-fold serially diluted with DMEM supplemented FBS + antibiotics from 2^0 to 2^{-10} (from 1 to 1/2048). Then, 100 µl of each dilution was added to six-replicated wells of the 96-well microplate seeded with the cell culture. DMEM solution was only added to cell control wells (medium + cell) and blank wells (only medium). The microplates were incubated at 37 °C and 5% CO2 for 24 h. The inert microscopy (Olympus ix71, Tokyo, Japan) was used to observe the morphological changes of the cells before the MTT assay.

Determination of cell viability

The MTT assay was used to determine the cell viability and inhibition by spectrophotometry. MTT solution was prepared at 5mg/ml concentration in PBS, filtered by a syringe filter (sterile, 0.22 µm) and stored at -20 °C.

After the incubation of 24 h, the sample solution in wells was discarded all and 50µl of MTT solution was added to all wells. The microplates were gently shaken and incubated for 4 hours at 37 °C in 5% CO2. The solvent was discarded after incubation. 50 µl of DMSO was added and the microplates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader (Absorbance 96, Byonoy, Germany) at a wavelength of 570 nm.

Data analysis

The percentage of cell viability and inhibition was calculated in Table 2, Figure 1 and 2 using the formula as following,

Cell viability (%) = $(A_{sample} - A_{blank})/(A_{control} - A_{blank}) \times 100$

Cell inhibition (%) = $100 - [(A_{sample} - A_{blank})/(A_{control} - A_{blank}) \times 100]$

A_{sample} = absorbance value of test compound, $A_{control}$ = absorbance value of control (cell), A_{blank} = absorbance value of blank (medium)

The means of data and the standard deviations (SD) were calculated for each group using SPSS 21 software (SPSS Inc., IL, USA). The effects on cell viability were analysed by one-way analysis of variance (ANOVA). Differences among absorbance data means of cell control and propolis experiments were compared using the Tukey post hoc test at a $P < 0.05$ level of significance. The linearity plots were demonstrated by Excel 2016 (Microsoft, USA). The half maximal cytotoxic concentration (CC50) of all products was calculated using the slope-intercept equations as "y = mx + b" in each figure (Fig. 1 and 2).

RESULTS

The OD absorbance data means and cell viability results of each products were calculated and compared with the results of their own cell controls and blanks in each microplates.

All propolis supplements had shown significant inhibition effects on the viability of both cell lines at different concentrations. When compared with the cell controls, P1, P2, P3, P4, P5 and P6 affected significantly the viability of BHK-21 cells up to the dilutions of 1/128, 1/16,

1/32, 1/16, 1/1 and 1/1, respectively ($p<0.05$ and $p<0.01$, Table 1). Both P2 and P4 products affected the BHK-21 cells approximately at the dilution ratio of 1/16. But, the highest inhibition was determined in P1 (93%) and P3 (91%) ($p<0.01$) and the lowest was observed in P6 ($p<0.05$) on BHK-21 cells (Figure 1). Figure 1 showed that there was a strong linear relation between the % cell inhibition and concentrations of P5 and P6 (R^2=0.980 and R^2=0.898 respectively), however, weak linearity for P1, P2, P3 and P4. The CC50s of P1, P2, P3, P4, P5 and P6 were calculated as 0.003, 0.178, 0,082, 0.451, 0.278 and 0.384 % for BHK-21, respectively.

Table 1. The description of propolis products used in the study

Products	Solvent	Form	Propolis Content (%)	Other Contents	Origin
P1	Water	Liquid	6 %	Honey, menthol, glycol	Turkey
P2	Water	Liquid	15 %	Glycol	Turkey
P3	-No data-	Liquid	-No data-	-No data-	Turkey
P4	Water	Liquid	15 %	Glycol	Turkey
P5	Water	Liquid	23 %	Honey, glycerol, licorice, eucalyptus aroma	Brasil
P6	Water	Liquid	5 %	Organic propolis	Turkey

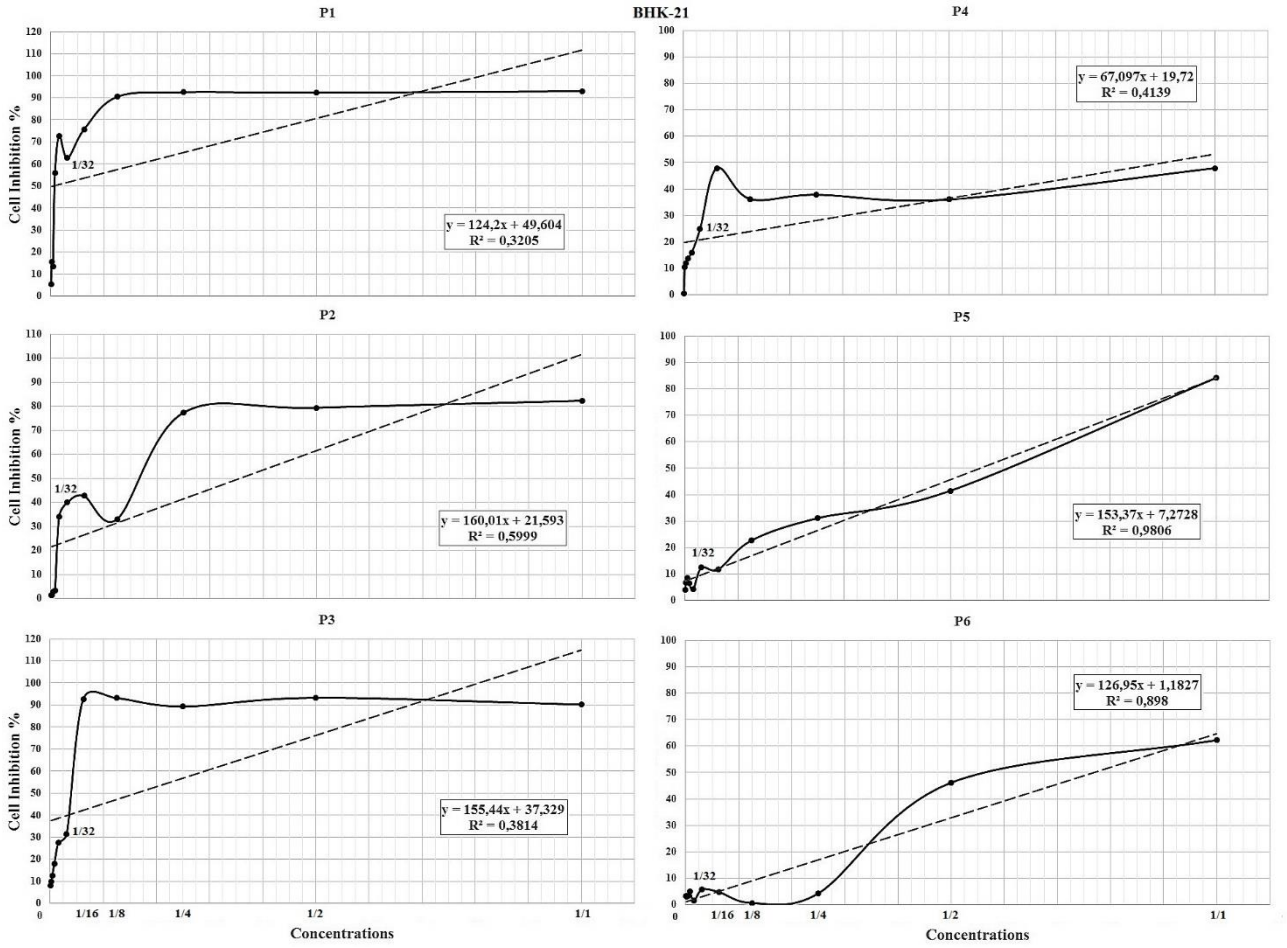

Figure 1. The inhibition effect of six propolis food supplements on BHK-21 cell line

On the viability murine macrophage cells (RAW 264.7), all propolis supplements had shown significant inhibition effects at the different dilution factors. When compared with the cell controls, P1, P2, P3, P4, P5 and P6 affected significantly the viability of macrophage cell line

up to the dilutions of 1/2, 1/8, 1/64, 1/8, 1/2 and 1/2, respectively ($p<0.05$ and $p<0.01$, Table 2). Both P2 and P4 products adversely affected the RAW 264.7 cells at the dilution ratio of 1/8 ($p<0.05$). But, the % cell inhibition increased in P3 despite of decreasing its concentration up to 1/64 ($p<0.01$). The highest % cell viability was significantly determined in P6 (49% on 1/2 dilution) ($p<0.05$, Table 2). Figure 2 showed that there was a strong linear relation between the % cell inhibition and concentration of P1, P5 and P6 (R^2 = 0.809, R^2=0.816 and R^2=0.944 respectively), however, weak linearity for P2, P3 and P4. The CC50 of P1, P2, P3, P4, P5 and P6 were calculated as 0.260, 0.218, 0.115, 0.257, 0.207 and 0.265% for RAW 264.7, respectively.

Table 2. The effects of six propolis products on viability of BHK-21 cell line

Dilutions	P1		P2		P3		P4		P5		P6	
	Abs. ± SD (nm)	**Cell Viability (%)**	**Abs. ± SD (nm)**	**Cell Viability (%)**	**Abs. ± SD (nm)**	**Cell Viability (%)**	**Abs. ± SD (nm)**	**Cell Viability (%)**	**Abs. ± SD (nm)**	**Cell Viability (%)**	**Abs. ± SD (nm)**	**Cell Viability (%)**
1/2	**0.137 ± 0.057**	7.0	**0.190 ± 0.033**	17.8	**0.151 ± 0.175**	9.8	**0.610 ± 0.090**	52.1	**0.171 ± 0.020**	15.9	**0.335 ± 0.060**	37.8
1/4	**0.147 ± 0.064**	7.7	**0.214 ± 0.154**	20.7	**0.113 ± 0.180**	6.7	**0.742 ± 0.282**	63.9	0.551 ± 0.088	58.5	0.468 ± 0.016	53.9
1/8	**0.144 ± 0.043**	7.5	**0.231 ± 0.171**	22.7	**0.162 ± 0.307**	10.7	**0.722 ± 0.136**	62.1	0.643 ± 0.256	68.9	0.815 ± 0.352	95.7
1/16	**0.176 ± 0.084**	9.5	0.603 ± 0.177	67.0	**0.115 ± 0.097**	6.9	**0.740 ± 0.151**	63.8	0.719 ± 0.101	77.3	0.846 ± 0.514	99.3
1/32	**0.409 ± 0.422**	24.3	0.522 ± 0.195	57.4	**0.119 ± 0.085**	7.2	**0.610 ± 0.058**	52.1	0.816 ± 0.394	88.3	0.811 ± 0.150	95.2
1/64	**0.613 ± 0.719**	37.3	0.545 ± 0.256	60.1	**0.875 ± 0.086**	68.6	0.865 ± 0.125	75.0	0.810 ± 0.344	87.6	0.803 ± 0.308	94.3
1/128	**0.456 ± 0.413**	27.3	0.595 ± 0.226	66.1	0.923 ± 0.135	72.5	0.965 ± 0.113	83.9	0.883 ± 0.368	95.8	0.839 ± 0.327	98.5
1/256	**0.719 ± 0.305**	44.0	0.852 ± 0.137	96.7	1.041 ± 0.156	82.1	0.991 ± 0.039	86.3	0.863 ± 0.239	93.6	0.810 ± 0.510	95.0
1/512	1.389 ± 0.261	86.6	0.856 ± 0.217	97.2	1.108 ± 0.213	87.5	1.010 ± 0.217	88.0	0.845 ± 0.123	91.5	0.823 ± 0.428	96.7
1/1024	1.354 ± 0.425	84.4	0.867 ± 0.127	98.5	1.142 ± 0.096	90.2	1.027 ± 0.239	89.5	0.861 ± 0.143	93.3	0.825 ± 0.139	96.9
1/2048	1.514 ± 0.594	94.6	0.868 ± 0.497	98.6	1.162 ± 0.066	91.9	1.137 ± 0.095	99.4	0.885 ± 0.217	96.0	0.825 ± 0.201	96.8
Cell Control	1.599 ± 0.063	100.0	0.880 ± 0.201	100.0	1.262 ± 0.095	100.0	1.144 ± 0.054	100.0	0.920 ± 0.112	100.0	0.851 ± 0.114	100.0
CC50 % in dilution)	0.003		0.178		0.082		0.451		0.278		0.384	

Table 3. The effects of six propolis products on viability of BHK-21 cell line

Dilutions	P1		P2		P3		P4		P5		P6	
	Abs. ± SD (nm)	Cell Viability (%)	Abs. ± SD (nm)	Cell Viability (%)	Abs. ± SD (nm)	Cell Viability (%)	Abs. ± SD (nm)	Cell Viability (%)	Abs. ± SD (nm)	Cell Viability (%)	Abs. ± SD (nm)	Cell Viability (%)
1/1	0,710 ± 0,057	29,6	0,178 ± 0,030	8,7	0,647 ± 0,175	25,1	0,517 ± 0,134	33,8	0,459 ± 0,034	14,7	0,295 ± 0,047	11,724
1/2	0,540 ± 0,064	22,5	0,918 ± 0,318	48,5	0,570 ± 0,180	22,1	0,668 ± 0,265	44,3	0,505 ± 0,044	16,3	1,182 ± 0,135	49,632
1/4	1,501 ± 0,043	62,5	0,943 ± 0,181	49,9	0,490 ± 0,307	19,0	0,770 ± 0,405	51,4	1,559 ± 0,380	52,5	1,456 ± 0,154	61,343
1/8	1,828 ± 0,084	76,1	0,604 ± 0,089	31,6	0,248 ± 0,097	9,6	0,572 ± 0,053	37,6	2,078 ± 0,858	70,3	2,333 ± 0,584	98,823
1/16	1,964 ± 0,422	81,8	1,589 ± 0,124	84,6	0,554 ± 0,085	21,5	1,139 ± 0,577	77,0	2,115 ± 0,729	71,6	2,219 ± 0,429	93,923
1/32	2,187 ± 0,719	91,1	1,530 ± 0,772	81,5	1,351 ± 0,086	52,4	1,217 ± 0,120	82,4	2,624 ± 0,745	89,1	2,203 ± 0,709	93,253
1/64	2,293 ± 0,413	95,5	1,738 ± 0,327	92,7	1,423 ± 0,135	55,2	1,222 ± 0,282	82,7	2,405 ± 0,438	81,5	2,227 ± 0,723	94,265
1/128	2,241 ± 0,305	93,3	1,823 ± 0,399	97,2	2,245 ± 0,156	87,1	1,185 ± 0,290	80,1	2,509 ± 0,749	85,1	2,200 ± 0,453	93,128
1/256	2,276 ± 0,261	94,8	1,867 ± 0,407	99,6	2,478 ± 0,213	96,1	1,105 ± 0,303	74,6	2,916 ± 0,234	99,1	2,140 ± 0,345	90,561
1/512	2,332 ± 0,425	97,1	1,827 ± 0,669	97,5	2,521 ± 0,096	97,8	1,319 ± 0,272	89,4	2,915 ± 0,628	99,0	2,357 ± 0,586	99,821
1/1024	2,321 ± 0,594	96,7	1,847 ± 0,424	98,5	2,508 ± 0,066	97,3	1,404 ± 0,397	95,4	2,937 ± 0,068	99,8	2,360 ± 0,287	99,963
Cell Control	2,401 ± 0,032	100,0	1,874 ± 0,180	100,0	2,578 ± 0,547	100,0	1,471 ± 0,291	100,0	2,943 ± 0,054	100,0	2,361 ± 0,180	100,000
CC50 % in dilution)	0,260		0,218		0,115		0,257		0,207		0,265	

DISCUSSION

Many studies suggested that propolis could have biological effects such as antibacterial, anti-inflammatory, tumorocidal and immunomodulator (Bogdanov, 2014; Bedier et al. 2016). The studies have focused on the effects of propolis on fibroblast cell lines such as BHK-21, gingival, skin and retinal because these cell lines exhibit fibroblast morphology for many studies (Wardati et al., 2014; Kartika et al., 2015, Bedier et al. 2016; Kurniati et al., 2018; Widjiastuti et al., 2020).

Ethanolic extract of propolis (100µg/ml, approx. 0.01%) with mineral trioxide aggregate increased the viability of BHK-21 cells for 24 h, however, the cell line was not affected by the propolis supplementation at 72 h and 7 days (Bedier et al. 2016). The propolis extracts and products such as oral gel had shown more biocompatibility and protective effects on BHK-21, odontoblastic and foreskin fibroblast cells against the cytotoxic effects of H2O2 (Aliyazicioglu et. al., 2011; Wardati et al., 2014; Kurniati et al., 2018). Human periodontal ligament (PDL) fibroblast cells were preserved

by propolis at up to 50 ug/ml (approx. %0,005) concentrations for 24 h. (Al-Haj Ali, 2016). Murase et al. (2013) determined that the water extract of Brazilian green propolis increased the viability of mouse retinal or human skin fibroblast cells against the UVA-induced cell damage. Likewise, Turkish propolis ethanolic extract showed dose-dependly an antioxidant activity on human fibroblast cells (Misir et al., 2018).

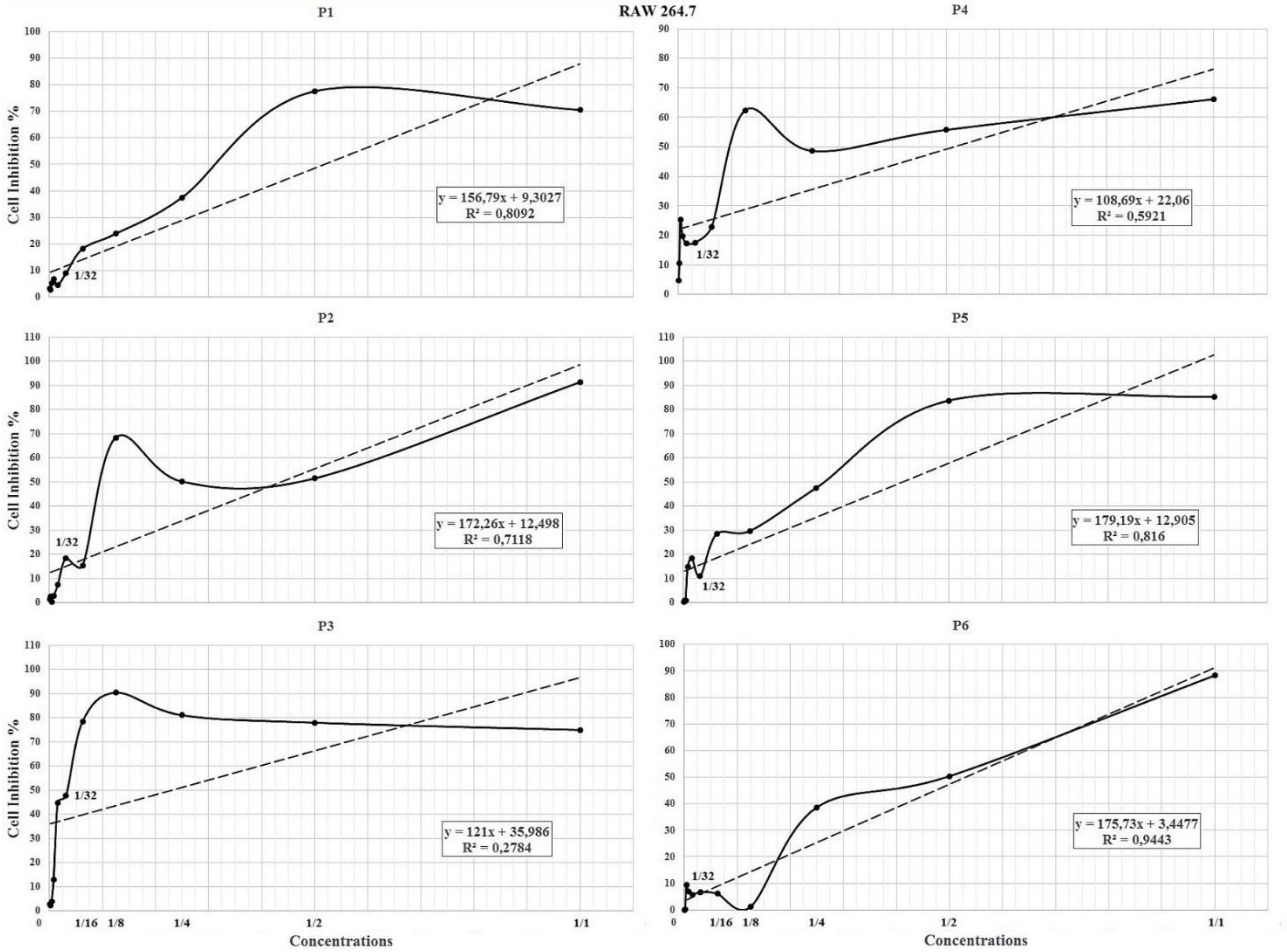

Figure 2. The inhibition effect of six propolis food supplements on RAW 264.7 cell line

Al-Haj Ali et al. (2016), Mooduto et al. (2018) and Ugur Aydın et al. (2018) suggested propolis were cytotoxic respectively at the concentration of 50 ug/ml (approx. 0.005%), 92.70 µg/ml (approx. 0,00927%) or greater and 15% (in ethanol) for gingival fibroblast cells. Likewise, Kartika et al. (2015) observed that 20ug/ml (approx. 0,002%) propolis decreased the % viability of dental pulp fibroblast from 80% to 32% (Widjiastuti et al., 2020). However, most of the studies have generally introduced that propolis and its extracts have low genotoxic, cytotoxic effects and are biocompatible on fibroblast cell lines (Wardati et al., 2014; Kartika et al., 2015; Al-Haj Ali et al., 2016; Kurniati et al., 2018; Mooduto et al., 2018). In this study, some propolis products contained flavouring, sweetener and aroma (such as menthol, glycol, liconice, eucalyptus) while some did not contain any additive. So, organic propolis and propolis products with only glycol had higher the CC50 and high viability of BHK-21 cells.

Propolis at 150ug/mL (approx. 0.015%) and 200ug/mL (0.02) reduced the viability of RAW 264.7 but propolis at 6.25–50ug/mL did not affect and was not toxic on RAW 264.7 cells when compared negative cell control (Sahlan et al., 2021).

Han et al. (2002) suggested that the water extract of Korean Propolis from 2.5 pg/ml to 25 pg/ml had no toxic effect on RAW 264.7.

However, Kim et al. (2019) observed that ethanolic extract of South Korean propolis decreased dose-dependently (10 to 40 ug/ml) the viability of RAW 264.7 independently of region. Asgharpour et al. (2019) determined the CC50 of ethanol extract of propolis as 15±3.2 µg/ml (approx. 0.0015%) for RAW 264.7. when compared water and methanolic extracts of Brazilian propolis, the water extract was lower toxic and had higher CC50 levels on murine macrophage-like J774 cells (Myint 2003). In this study, the CC50s of the products were higher for RAW 264.7 cells than for BHK-21 cells. So, low cytotoxic effect was determined in RAW 264.7 cells even at high concentrations of the propolis products.

CONCLUSION

Despite their rich bioactive compound content, the propolis products affected both baby hamster kidney cells and murine macrophage cells at different concentrations. The results of many studies suggested that alcoholic extracts of propolis or containing other solvents and additives adversely affected the cell viability. Similarly, the results of this study introduced that the dilution factors and additives might be cytotoxicity-determining factor of the propolis products.

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Investigation of correlation between corneal thickness and intraocular pressure in New Zealand Albino Rabbits

Research Article

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ABSTRACT

The aim of this study was to determine intraocular pressure (IOP) and central corneal thickness (CCT) measurements in healthy rabbits to establish clinical reference values and to investigate the possible relationship between these measurements. The study included 40 eyes of 20 New Zealand albino rabbits, aged 1.5-2 years. All the eyes were healthy with no abnormalities, corneal disease, or evidence of glaucoma. An ultrasonographic pachymeter was used to measure CCT and TonoVet® was used to measure IOP. Correlations between IOP and CCT measurements were examined. The mean CCT was 388.2 ± 38.22 µm in the right eye and 391.8± 59.18 µm in the left eye. IOP was measured as 16 ± 3.76 mmHg in the right eye and 16 ± 2.73 mmHg in the left eye. No correlation was determined between the IOP and CCT, and this indicated that the TonoVet® readings of CCT and IOP did not cause a deviation that could be determined. There is a need for further studies of different animals to investigate the effect of corneal thickness on the IOP measurements made with TonoVet®.

Keywords: Central corneal thickness, pachymeter, rebound tonometer, tonovet**.**

INTRODUCTION

Glaucoma is the most important cause of permanent blindness in humans and domestic animals. Degeneration in the optic nerve head and retina forms with an increase in intraocular pressure (IOP) (Garway-Heath et al., 2015; Gelatt & MacKay, 2004a, 2004b; Tham et al., 2014). IOP is accepted as one of the greatest risk factors for the development of glaucoma, and is the most consistent predictor of glaucoma damage in both humans and animals. Accurate measurement and follow up of IOP is important for the diagnosis of glaucoma and treatment follow-up. Therefore, the available treatments for this disease focus on first reducing IOP (Gloe et al., 2019). Rabbits have been used for many years in glaucoma studies, and these studies have contributed to the development of drugs designed to reduce IOP, and to surgical procedures and medical devices. Rabbits are relatively inexpensive, and the care and study of large numbers is simple. Moreover, their clinical importance has increased in recent years as they are preferred as domestic pets (Millar & Pang, 2014; Hong Zhang et al., 2014).

Other types of tonoometry used in rabbits include Schiøtz (Becker, 1960; Behar-Cohen et al., 1996), MacKay-Marg (Wind & Irvine, 1969), Perkins (Acosta et al., 2007), Draeger (Kass et al., 1972), GAT (Jin et al., 2014), the Tono-Pen® ve TonoPen-XL® (Gerometta et al., 2012; Ito et al., 2013) and airpuff (Gupta et al., 2007).

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It is known that biomechanical properties such as central corneal thickness (CCT) and fluid content affect IOP, and correct measurement of IOP is important to understand how it is affected by these factors. There is evidence that there is a relationship between glaucoma and properties related to the cornea such as CCT (Belovay & Goldberg, 2018; Brandt et al., 2004; Brown & Congdon, 2006).

As there are variations in CCT between individuals, a great variation in CCT can cause an incorrect estimation of IOP and may cause incorrect diagnosis in the classification of glaucoma (Mansoori & Balakrishna, 2018). Therefore, it is important to determine how various tonometers are affected by CCT.

The estimated IOP values will likely be dependent upon corneal surface conditions, such as the central corneal thickness (CCT), the corneal curvature, and the precorneal tear film. Nearly all human studies have shown that the CCT is positively correlated with IOP (Broman et al., 2007; Harada et al., 2008; Zeng et al., 2008). However, the effects of other corneal factors are still in dispute. The degree that the CCT affects IOP nevertheless varies between each study and tonometer (Bhan et al., 2002; Iliev et al., 2006). In the literature reviews, it was determined that the applanation tonometer was mostly used in the studies (Cairns et al., 2019; Moussa et al., 2021; Sethi et al., 2021; Hui Zhang et al., 2020). In the present study, it is also among the aims to investigate the effect of measurement with rebound tonometry. The aim of this study was to measure IOP and CCT in healthy rabbits and to determine whether or not there was any correlation between these measurements.

MATERIAL and METHOD

The study sample comprised 20 adult albino rabbits (10 males, 10 females), aged 1.5-2 years. In the physical and ophthalmoscopic examinations, the whole eye was evaluated with additional organs. All the eyes of all the rabbits were healthy with no disorder in the cornea and anterior/posterior chamber, or findings of glaucoma.

An ultrasonographic pachymeter (Pocket II, Quantel) was used in the measurement of both cornea thicknesses in all the rabbits. When taking the measurement, the probe was placed vertical to the cornea and gentle contact was made. The measurement result was obtained as the mean of 5 measurements taken by the device. The corneal thickness was recorded as µm separately for each eye. In all the rabbits, IOP was measured using a TonoVet® (icare) rebound tonometry device, which provides the average of 5 measurements displayed on a digital screen. The measurements obtained were recorded separately for each eye (Figure 1). No anesthetic agents were used during the examination and measurements. All the measurements were gathered between 02:00 pm and 04:00 pm by the same veterinary surgeon.

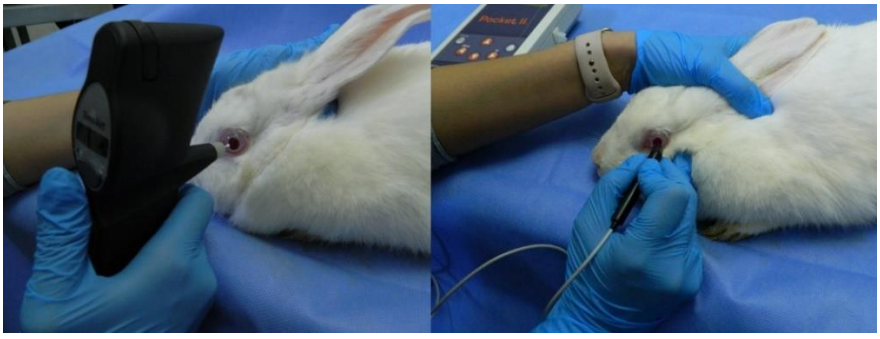

Figure 1. IOP and CCT measurements.

Statistical Analysis

Data obtained in the study were analyzed statistically using SPSS vn. 14.01 software (SPSS Inc, USA). Descriptive analyses were used to summarize data and to check if assumptions were met. The results were evaluated using the Shapiro Wilk Test for normality, and the Levene Test for homogeneity of variances. Differences in the IOP and CCT measurements between the right and left eyes were assessed with Independent Samples T-Test. The Pearson correlation coefficient was computed to evaluate relationships. A value of $p<0.05$ was considered statistically significant for all analyses.

RESULTS

The mean CCT was 388.2 ± 38.22 µm in the right eye and 391.8± 59.18 µm in the left eye. IOP was measured as 16 ± 3.76 mmHg in the right eye and 16 ± 2.73 mmHg in the left eye (Table 1).

Table 1. Mean value of IOP and CCT in right and left eyes.

		N	Mean	SD	P
IOP	Right	20	16	3,76	1
	Left	20	16	2,73	
CCT	Right	20	388,2	38,22	0,818
	Left	20	391,8	59,18	

The IOP and CCT values of the right and left eyes were examined separately with the Paired Samples t-test. No significant difference was determined.

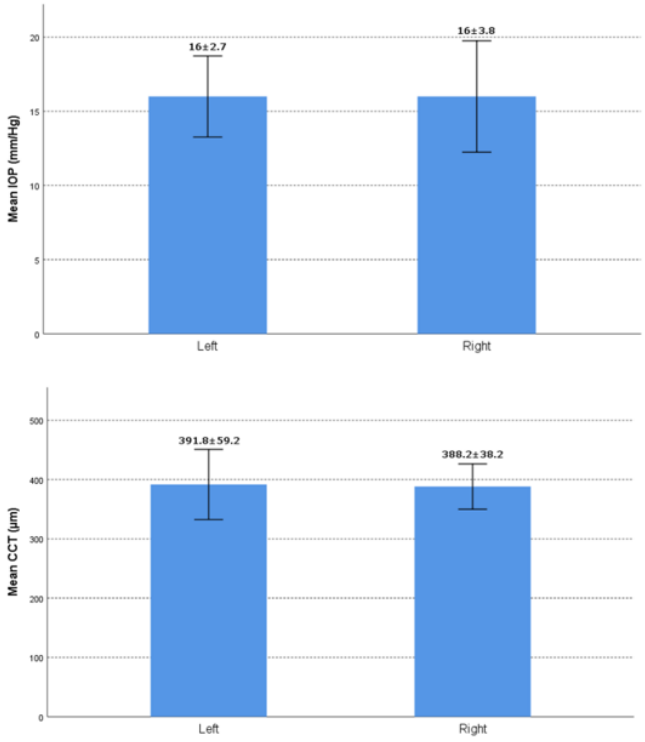

Figure 2. The bar graphs show the IOP and CCT values of the left and right eye.

Correlations between IOP and CCT were examined in the right eye (p=0.961, r=0.012), the left eye (p=0.435, r=0.185) and both eyes together (p=0.097, r=0.553) and no significant correlation was determined (Figures 3, 4).

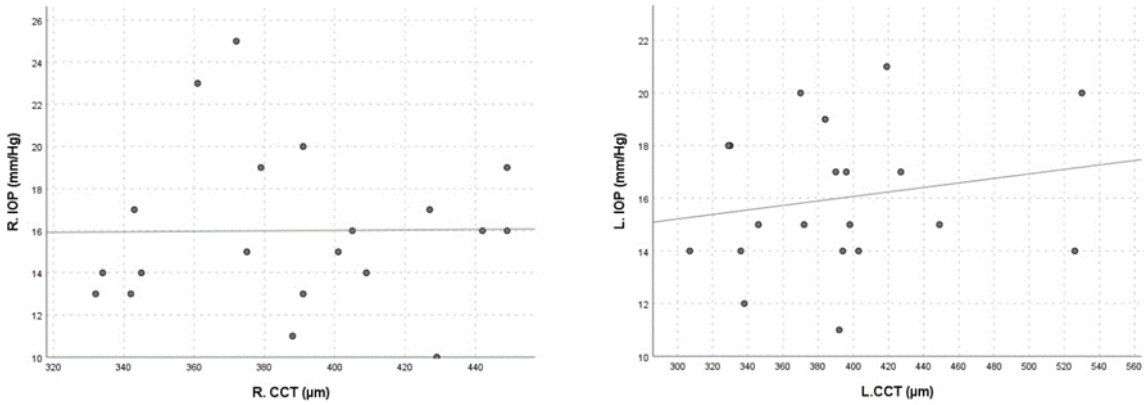

Figure 3. Scatter Dot Plot of IOP and CCT, right and left eyes (R: Right eye, L: Left eye, CCT: Central corneal thickness, IOP: intraocular pressure)

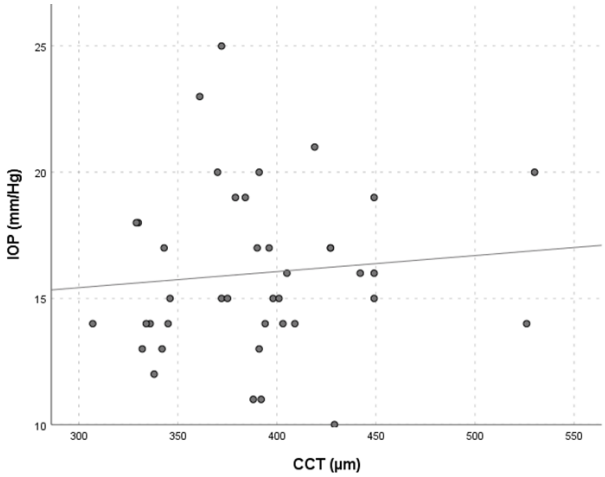

Figure 4. Scatter Dot Plot of IOP and CCT, both eyes (CCT: Central corneal thickness, IOP: intraocular pressure.

DISCUSSION

Rabbits are widely used as animal models in pharmacological tests for the examination of glaucoma and ocular diseases, and have recently been increasingly owned as domestic pets (A. Bouhenni et al., 2012; Hong Zhang et al., 2014).

An accurate, repeatable method for the measurement of IOP in rabbits is of vital importance. IOP is known to be affected by many types of sedative and general anaesthetic agents. In a previous study it was stated that if sedation or general anaesthesia is applied, attention must be paid not only to the effects of the agents used but also to how the effect changes in the time following application. Overall, general anaesthetics (injectable or gas) significantly reduce basic IOP (Millar & Pang, 2014). Therefore, in the current study, no anaesthetic or sedative agent was used.

During an ophthalmic examination, clinicians measure and record values in the patient file such as the amount of tears, IOP, and corneal thickness. This is necessary for individual follow-up of the patient and for the formation of

type-specific data records. It is clear that studies to determine reference values are important in the fields of both experimental and clinical studies. Species-specific studies can be considered to contribute to literature to be able to clearly establish reference values. In the current study, the mean values of IOP were determined with TonoVet® and mean CCT values with pachymetry in healthy New Zealand albino rabbits.

From a literature scan (Table 2) it was seen that IOP values ranged between 3mmHg and 80 mmHG, and when studies are examined in detail, the values can be seen to be mostly in the range of 9-15mmHg. The IOP values obtained in the current study were found to be similar to the findings of studies by Williams et al, Doğan and Kıbar, and Pereira et al(Doğan & Kibar, 2015; Pereira et al., 2011; Williams, 2012). Studies that have aimed to determine CCT values in rabbits have reported (Table 3) values ranging from 356µm to 407µm (Abrams et al., 1996; Charisis et al., 2008; Doğan & Kibar, 2015; Lim et al., 2005b; Ma et al., 2016; Mermoud et al., 1995; Pereira et al., 2011; Wang et al., 2013; Williams, 2012). The CCT values recorded in the current study were determined to be within this range. Consistent with the findings of previous studies, no significant difference was determined between the right and left eyes in respect of the IOP (Abrams et al., 1996; Charisis et al., 2008; Doğan & Kibar, 2015; Lim et al., 2005b; Ma et al., 2016; Mermoud et al., 1995; Pereira et al., 2011; Wang et al., 2013; Williams, 2012) and CCT (T. Chan et al., 1983; Herse & Yao, 2009; Khan, 2019; H. F. Li et al., 1997; Schulz et al., 2003; Wang & Wu, 2013) values.

Table 2. IOP values in literatures.

IOP	
16 ± 3,76 mmHg Right 16 ± 2.73 mmHg Left	Present study
3-30 mmHg	(Abrams et al., 1996) Abrams ve ark, 1996
0-50 mmHg	(Lim et al., 2005a)Lim ve ark, 2005
11.06 ± 1.62 mmHg	(Ma et al., 2016)Ma ve ark, 2016
9.51 ± 2.62 mmHg TonoVet® 15.44 ± 2.16 mmHg Tono-Pen Avia	(Pigatto et al., 2011)Pereira ve ark, 2011
12.99±2.67 mm/Hg	(Doğan & Kibar, 2015)Doğan ve Kibar, 2015
10.25 ± 2.3 mmHg Right 9.07 ± 2.47 mmHg Left	(Wang et al., 2013)Wang ve ark, 2013
15-23 mm Hg Yenidoğan 25 -50 mm Hg 1- 3 aylık	(Williams, 2012)
5-80 mmHg (Tono-Pen XL)	(Mermoud et al., 1995) Mermoud ve ark, 1995
10-70 mmHg (Tono-Pen XL)	(Charisis et al., 2008) Charisis ve ark, 2008

Table 3. CCT values in literatures.

CCT	
388,2 ± 38,22 µm Right 391,8± 59,18 µm Left	Present Study
381.6 ± 27,3 µm	(H. F. Li et al., 1997) Li ve ark, 1997
407 ± 20 µm	(Toiloi Chan et al., 1983) Chan ve Holden, 1983
356.11 ± 14.34 µm	(Schulz et al., 2003) Schulz ve ark, 2003
540 ± 25 µm	(Herse & Yao, 2009) Herse ve Yao, 1993
387 ± 19.8 µm Right 384 ± 20.2 µm Left	(Wang & Wu, 2013) Wang ve Wu, 2013
Pachymeter 372.47±20.11µm Right 373.20±20.32 µm Left spectral-domain anterior segment optical coherence tomography	(Khan, 2019) Khan, 2019

375.40±20.12 µm Right 376.09±20.45 µm Left

CONCLUSION

Previous studies in literature have reported that Goldmann applanation tonometry is effective in the measurement of CCT values (J. Li, 2004; Nejabat et al., 2016; Ozbek et al., 2006). That there was no correlation between IOP and CCT in the current study shows that there was no deviation that could be determined in the TonoVet® readings of CCT and IOP. The determination that there was no effect of corneal thickness in the IOP measurements made with TonoVet® is extremely important information in both clinical terms and in respect of experimental studies. Nevertheless, there is a need for further similar studies of different animal species to support these results.

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Milk yield and milk quality characteristics of Awassi sheep under semi-intensive conditions

Research Article

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ABSTRACT

This study was carried out to determine the milk yield and milk quality parameters of Awassi sheep raised under semi-intensive conditions in Harran University Experimental Animal Research and Application Center. In the study, 2 and 3 years old, 46 heads of Awassi sheep were used as animal material. The ewes were cared for and fed in a manner routinely used at the Animal Research and Application Center. Average daily milk yield in Awassi sheep was determined as 1002.82±52.57 g. Lactation milk yield and lactation period were calculated as 168.10±8.44 kg and 166.10±2.11 days, respectively. In the study, the overall mean fat, protein, lactose and dry matter ratios were determined. %6.27±0.10; 5.12±0.05; 4.81±0.05 and 17.44±0.13 respectively. Somatic cell count and pH values in Awassi sheep milk were determined as 207.56±21.29 cell/ml and 6.29±0.03 respectively. L*, a* and b* values in sheep milk were determined as 72.69±0.16; -6.11±0.04 and 6.92±0.08. respectively. It was determined that the amount of butyric acid (C4:0), which is one of the short-chain fatty acids, was higher than the values reported for sheep milk in all groups examined in the study. As a result, it has been determined that there is a wide variation in milk yield in Awassi sheep. A rapid genetic improvement may be achieved by utilizing this variation

Keywords: Awassi, milk, fatty acid profile, sheep, somatic cell count

INTRODUCTION

Sheep breeding is carried out in various regions in the world. It is the most profitable livestock farming in regions with steppe climatic conditions, arid zones, and wide grasslands. Sheep are easy to manage, and the cost of sheep breeding is lower than other livestock husbandry practices. They are capable of using vegetation that cattle cannot make use of sheep farming is an important source of livelihood in areas where agriculture is not well developed and feed resources are limited (Akçapınar, 2000; Akçapınar et al., 2002).

Considering the geographical and climatic characteristics of Turkey, sheep breeding, which is one of the major livestock practices, is widely carried out in almost every region of this country. Especially in the Eastern and Southeastern Anatolia regions, sheep breeds constitute the majority of animal husbandry (TUİK, 2020). Sheep breeding in Turkey has combined production level including meat, milk, and wool. The yield of these products varies according to the breed and is often low; however, Awassi is an important breed for milk production (Akçapınar, 2000; Akçapınar and Özbeyaz, 1999).

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Awassi is generally grown in the Southeastern Anatolia region of Turkey. The Awassi breed, called the Arabian sheep, originates from the Mesopotamia region located between the Euphrates and Tigris rivers. It is widely raised in the Arab countries, Israel, and North Africa surrounded by the Mediterranean coast. It is raised in low altitude provinces with desert-like climatic conditions including Gaziantep, Şanlıurfa, and Hatay, along the Syrian border of Turkey (Akçapınar, 2000; Kaymakçı, 2010).

Due to the habits and demand for dairy products containing sheep milk in Turkey, sheep milk is an important source of income for breeders in some regions (Esen and Özbey, 2002). Therefore, it is important to reveal the current characteristics of yield performance of breeds for milk production by pure breeding and selection breeding. Milk obtained from Awassi sheep in the Southeastern Anatolia region and Şanlıurfa province is used in the production of several local dairy products and is highly preferred by consumers. It is greatly important to determine the quality parameters of these products, which have a large effect on regional promotion, during the process of registration of geographical indication (Oraman, 2015).

In this study, we aimed to determine the milk yield and milk quality parameters of Awassi sheep raised under semi-intensive conditions in Harran University Research and Application Center for Experimental Animal.

MATERIAL and METHOD

Animal material

The study was conducted in the Livestock Unit of Harran University Research and Application Center for Experimental Animal (Harran /Turkey). A total of 46 Awassi ewes aged 2 and 3 years with single lamb were included.

Care and feeding

The care and feeding of sheep was performed as routinely followed by the sheep breeding unit, considering the pasture and climatic conditions. The sheep were fed in the barn during the lambing period. During the spring pasture period, the animals were not released into the pasture in the morning until the frost cleared, and they were kept in the shelter at night. They were fed with concentrated feed (18% CP, 2600 kcal/kg) and wheat straw in the evening when they returned to the pasture.

Milk yield and quality

To determine the milk yield and characteristics of milk quality, 46 ewes aged 2 and 3 were used. Ewes were selected on the days when the births increased, and care was taken to keep the date of lactation onset close to each other. Milk controls were started on day 15 after birth on average, and monthly controls were continued until the milk yield reduced to below 100 g. Milking was performed using semiautomatic mobile (Sezer) milking machine at around six in the morning and evening. On control days, the lambs were separated from their mothers at 18:00 on the previous day and left until after milking the next day. The morning and evening milk yields in the control milking were summed and the milk yield on the control day was calculated. The 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, and 180-day milk yields were determined from the milk yield records using the interpolation method. The calculated values were used to determine the lactation milk yield using the Trapeze II method (Fleischmann Method) (Ünal et al., 2008).

To determine the characteristics of milk quality, milk samples from each age group were collected four times during the early (mean 45th day), middle (mean 75th and 105th days), and late (mean 135th days) of lactation. A total of 100 mL of milk samples obtained from morning milking were used for the related analysis. Dry matter, fat, protein, and lactose ratios and somatic cell count (SCC) were determined at

Istanbul University Faculty of Veterinary Medicine using the Bentley 150 Combi Milk Analyzer. The pH and color of the milk samples were determined using a portable pH meter and colorimeter, respectively, immediately after milking (Doğan and Boztepe, 2012; Priolo et al., 2003). Milk samples collected in the morning from eight randomly selected sheep from each age group on day 75 of lactation were used to detect fatty acid composition. For this purpose, 5 mL of hexane was added to 5 mL of milk and vortexed for 5 min. The resulting mixture was centrifuged at 4000 rpm for 15 min and kept in a dark room at +4°C for 24 h. Then, the clear hexane-containing supernatant was transferred to eppendorf tubes and brought to the Harran University Science and Technology Research Center in the cold chain. Milk fat samples were dissolved in 10 mL of hexane, and 0.5 mL of 2 N KOH–methanol solution was added, and then shaken in a vortex and kept in the dark for 1 h. The supernatant gas layer was sampled and read in the GC FID device (GC-FID: Shimadzu nexis GC 2030, Colon: Teknokroma tr882192 Capillary Column TR - CN 100).

Statistical analyzes

Independent sample t-test was used to compare age groups. SPSS package program was used for calculations (IBM SPSS 20.0 for Windows).

RESULTS

The statistical values of daily milk yield, additive milk yield, lactation milk yield, and lactation period in sheep at different periods of lactation are shown in Tables 1, 2, and 3. The difference between the age groups on day 105, 120, 135, and 150 of lactation was statistically significant ($P<0.05$ and $P<0.01$). Significant differences were observed in the groups in terms of added milk yield on day 165 and 180 of lactation ($P<0.05$). The lactation curve drawn according to the average daily milk yield of the sheep examined within the scope of the research is shown in Figure 1. The parameters of milk quality are shown in Table 4. No significant difference was observed in the quality parameters between the 2- and 3-year-old animal groups, except for the pH value on day 45. The fatty acid ratios determined in sheep milk and the index values calculated from these values are shown in Table 5.

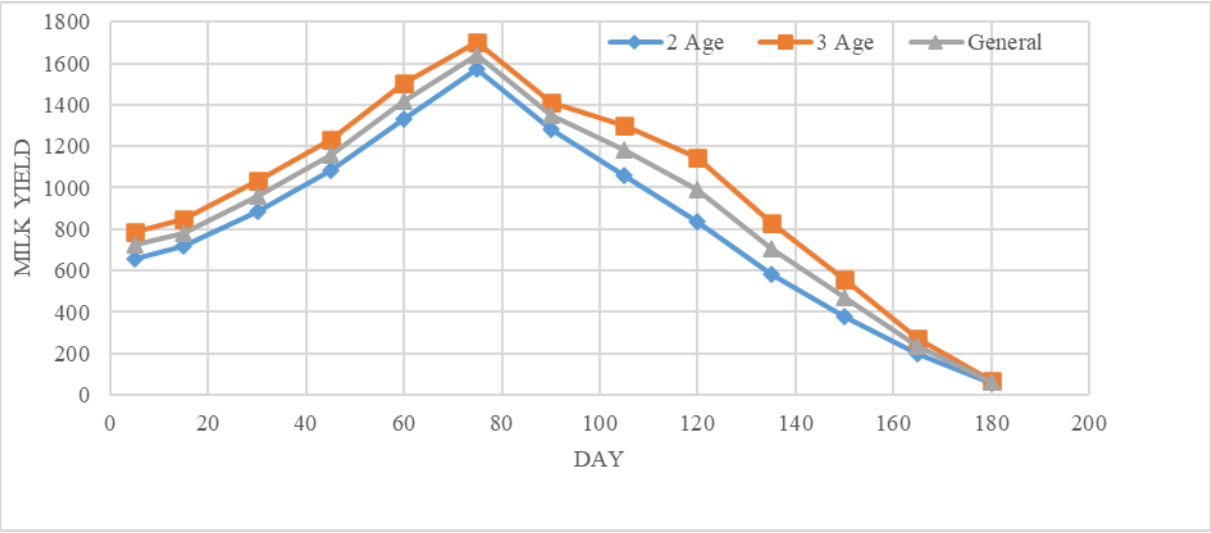

Figure 1. Lactation curve in Awassi sheep.

Table 1. Statistical values of daily milk yield in various days of lactation in Awassi sheep (g)

Age		Day 15	Day 30	Day 45	Day 60	Day 75	Day 90	Day 105	Day 120	Day 135	Day 150	Day 165	Day 180	Daily Milk Yield
2	**n**	23	23	23	23	23	23	23	23	22	19	14	6	23
	X±SE	715.34±112.44	883.87±106.49	1084.05±106.88	1333.97±95.44	1575.18±108.41	1279.06±86.32	1059.28±76.7[a]	834.03±75.84[a]	579.62±66.45[a]	379.81±49.1[a]	198.09±37.30	57.16±9.29	937.97±75.85
	Min	39.35	141.71	244.06	278.45	361.50	355.50	328.30	232.37	28.87	82.80	28.00	15.33	215.30
	Max	2028.25	2198.88	2369.50	2273.33	2710.75	2026.23	1721.80	1565.80	1213.50	831.00	448.50	76.50	1889.99
	%V	75.38	57.78	47.28	34.31	33.01	32.37	34.74	43.61	53.77	56.29	70.46	39.80	38.78
3	**n**	23	23	23	23	23	23	23	23	23	20	17	9	23
	X±SE	848.52±121.38	1034.83±112.74	1232.98±110.42	1501.73±97.37	1703.07±120.64	1414.41±90.39	1301.21±82.8[b]	1145.94±87.0[b]	827.08±74.93[b]	556.6±54.96[b]	271.43±39.08	66.83±3.92	1067.67±71.90
	Min	58.29	300.06	314.18	516.13	539.25	417.53	404.53	366.60	260.10	153.60	34.87	56.27	353.19
	Max	1914.00	2159.07	2388.63	2485.19	2779.00	2103.93	1969.43	1981.97	1505.47	1028.97	552.47	90.53	1566.56
	%V	68.60	52.25	42.95	31.09	33.97	30.65	30.50	36.43	43.45	44.15	59.36	17.59	32.30
P		NS	NS	NS	NS	NS	NS	*	**	*	*	NS	NS	NS
Total	**n**	46	46	46	46	46	46	46	46	45	39	31	15	46
	X±SE	781.96±82.40	959.35±77.50	1158.52±76.78	1417.85±68.56	1639.12±80.76	1346.74±62.61	1180.25±58.64	989.99±61.63	706.1±53.04	470.5±39.18	238.31±27.63	62.96±4.38	1002.82±52.57
	Min	39.35	141.71	244.06	278.45	361.50	355.50	328.30	232.37	28.87	82.80	28.00	15.33	215.30
	Max	2028.25	2198.88	2388.63	2485.19	2779.00	2103.93	1969.43	1981.97	1505.47	1028.97	552.47	90.53	1889.99
	%V	71.47	54.79	44.95	32.79	33.41	31.53	33.70	42.22	50.39	52.01	64.55	26.95	35.55

a.b: The difference between the means with different letters in the same column is statistically significant (P<0.05).
NS: Nonsignificant (P>0.05); *: P<0.05; **: P<0.01

Table 2. Statistical values of additive milk yield in various days of lactation in Awassi sheep (kg)

Age		Day 30	Day 45	Day 60	Day 75	Day 90	Day 105	Day 120	Day 135	Day 150	Day 165	Day 180
2	**n**	23	23	23	23	23	23	23	22	19	14	6
	X±SE	22.73±3.31	37.48±4.86	55.62±6.23	77.44±7.57	98.85±8.79	116.38±9.59	130.58±10.03	140.1±10.22	147.51±10.33	150.76±10.41[a]	151.78±10.46[a]
	Min	1.95	4.84	8.76	13.56	18.94	24.07	28.80	32.61	34.78	35.61	35.82
	Max	62.13	96.39	131.21	168.59	204.12	228.16	239.51	242.01	242.01	242.01	242.01
	%V	69.93	62.18	53.69	46.87	42.65	39.52	36.83	34.99	33.59	33.13	33.04
3	**n**	23	23	23	23	23	23	23	23	20	17	9
	X±SE	26.85±3.54	43.86±5.09	64.37±6.46	88.41±7.88	111.79±9.214	132.16±10.24	150.51±11.12	165.31±11.84	175.14±12.39	180.28±12.78[b]	181.98±12.96[b]
	Min	5.04	13.29	21.58	31.60	38.78	44.94	50.73	55.43	58.53	60.04	60.39
	Max	57.57	86.88	123.43	162.70	198.63	226.96	250.41	268.73	282.07	290.41	293.75
	%V	63.15	55.61	48.11	42.72	39.53	37.17	35.44	34.34	33.93	34.01	34.15
P		NS	NS	NS	NS	NS	NS	NS	NS	NS	*	*
Total	**n**	46	46	46	46	46	46	46	45	39	31	15
	X±SE	24.79±2.42	40.67±3.51	60.00±4.48	82.92±5.46	105.32±6.37	124.27±7.04	140.55±7.55	153.15±7.94	161.32±8.24	165.52±8.44	166.88±8.53
	Min	1.95	4.84	8.76	13.56	18.94	24.07	28.80	32.61	34.78	35.61	35.82
	Max	62.13	96.39	131.21	168.59	204.12	228.16	250.41	268.73	282.07	290.41	293.75
	%V	66.09	58.54	50.67	44.67	41.02	38.41	36.44	35.17	34.63	34.60	34.69

Table 3. Statistical values of lactation milk yield and lactation period in Awassi sheep

Lactation Milk Yield (kg)				
Age	**n**	**X±SE**	**Min**	**Max**
2	23	152.84±10.36	36.96	237.93
3	23	183.37±12.76	61.65	297.15
P		NS		
Total	46	168.10±8.44	36.96	297.15
Lactation Period (Day)				
Age	**n**	**X±SE**	**Min**	**Max**
2	23	163.04±3.37	125.00	180.00
3	23	169.26±2.44	145.00	180.00
P		NS		
Total	46	166.15±2.11	125.00	180.00

Table 4. Milk quality characteristics of Awassi sheep (%)

			Day 45	Day 75	Day 105	Day 135	Total
Traits	**Age**	**n**	**X±SE**	**X±SE**	**X±SE**	**X±SE**	**X±SE**
Fat Percentage (%)	2	23	5.12 ±0.26	5.73±0.18	6.78±0.21	7.37±0.24	6.27±0.10
	3	23	5.01±0.20	6.16±0.14	6.80±0.25	7.21±0.23	
Protein Percentage (%)	2	23	4.69±0.12	4.90±0.09	5.59±0.12	5.48±0.13	5.12±0.05
	3	23	4.69±0.09	4.87±0.10	5.41±0.12	5.36±0.23	
Laktose Percentage (%)	2	23	5.25±0.04	5.26±0.04	4.69±0.10	4.19±0.15	4.81±0.05
	3	23	5.18±0.05	5.19±0.05	4.62±0.09	4.07±0.15	
Dry Matter Percentage (%)	2	23	16.42±0.31	17.28±0.23	18.33±0.30	18.09±0.40	17.44±0.13
	3	23	16.23±0.26	17.58±0.21	18.06±0.34	17.62±0.55	
Freezing Point	2	23	0.58±0.00	0.58±0.00	0.57±0.00	0.57±0.01	0.57±0.00
	3	23	0.57±0.00	0.58±0.00	0.57±0.00	0.56±0.01	
SCC (x10^3 cell/ml)	2	23	206.17±52.49	240.61±77.28	190.70±51.06	341.35±108.54	207.56±21.29
	3	23	187.17±48.68	175.74±34.46	139.43±25.75	178.78±36.15	
pH	2	23	6.70±0.02^a	6.55±0.01	6.02±0.06	5.89±0.05	6.29±0.03
	3	23	6.65±0.02^b	6.55±0.02	6.00±0.05	5.93±0.05	
L*	2	23	72.29±0.37	71.17±0.63	73.43±0.22	72.22±0.45	72.69±0.16
	3	23	73.16±0.37	72.51±0.65	73.83±0.24	72.92±0.41	
a*	2	23	-6.31±0.09	-6.67±0.12	-5.93±0.10	-5.83±0.09	-6.11±0.04
	3	23	-6.14±0.10	-6.41±0.11	-5.85±0.13	-5.73±0.13	
b*	2	23	6.62±0.17	6.39±0.17	6.83±0.20	7.61±0.28	6.92±0.08
	3	23	6.74±0.15	6.66±0.20	6.81±0.21	7.69±0.24	

a,b: The difference between the means with different letters in the same column is statistically significant (P<0.05).

Table 5. Milk fatty acid ratios and index values in Awassi sheep (%)

Fatty Acid	Age	n	X±SE
Butyric Acid (C4:0)	2	8	3.41±0.46
	3	8	4.02±0.1
Caproic Acid (C6:0)	2	8	3.60±0.27
	3	8	4.03±0.09
Caprylic Acid (C8:0)	2	8	3.97±0.17
	3	8	4.28±0.16
Capric Acid (C10:0)	2	8	13.83±0.7
	3	8	14.77±0.57
Undecanoic Acid (C11:0)	2	8	0.34±0.02
	3	8	0.35±0.03
Lauric Acid (C12:0)	2	8	7.23±0.41
	3	8	7.48±0.30
Myristic Acid (C14:0)	2	8	16.20±0.35
	3	8	16.67±0.48
Myristoleic Acid (C14:1)	2	8	0.27±0.01
	3	8	0.29±0.02
Pentadecanoic Acid (C15:0)	2	8	0.82±0.05
	3	8	0.92±0.05
Palmitic Acid (C16:0)	2	8	29.08±1.18
	3	8	28.48±0.98
Palmitoleic Acid (C16:1)	2	8	0.48±0.03
	3	8	0.43±0.04
Trans-Elaidic Acid (C18:1n9t)	2	8	3.91±0.23
	3	8	3.71±0.11
Stearic Acid (C18:0)	2	8	3.46±0.15
	3	8	3.26±0.24
Cis-Oleic Acid (C18:1n9c)	2	8	13.28±0.70
	3	8	11.51±0.62
Cis-Linoleic Acid (C18:2n6c)	2	8	0.75±0.07

	3	8	0.61±0.08
Linolenic Acid (C18:3n6)	2	8	0.23±0.01
	3	8	0.20±0.02
Total Saturated Fatty Acid	2	8	80.08±0.79[b]
	3	8	84.25±0.72[a]
Total Monounsaturated Fatty Acid	2	8	18.94±0.74[a]
	3	8	14.94±0.66[b]
Total Poliunsaturated Fatty Acid	2	8	0.98±0.08
	3	8	0.80±0.10
Total Unsaturated Fatty Acid	2	8	19.92±0.79[a]
	3	8	15.75±0.72[b]
Nutritional Value	2	8	0.69±0.03
	3	8	0.62±0.04
Atherogenic Index	2	8	3.78±0.25[b]
	3	8	5.02±0.34[a]

a.b: The difference between the means with different letters in the same column is statistically significant (P<0.05).

DISCUSSION

In this study, in addition to the lactation data of Awassi sheep, fatty acid profile, which is an important milk quality parameter for healthy nutrition in humans, and some characteristics of milk quality (dry matter, fat, protein, lactose ratios, SCC, and pH) were determined. The values of milk yield (Tables 1, 2, and 3) of 3-year-old Awassi sheep were higher. As milk yield in sheep is a characteristic that increases with age (Akçapınar, 2000; Akçapınar and Özbeyaz, 1999; Kaymakçı, 2010), high yields are expected in the elderly group. In the present study, the average daily milk yield for Awassi sheep was higher than that reported in studies conducted in Konya Ereğli (Yalçın and Aktaş, 1969) and in Awassi sheep raised in the Duhok region in the north of Iraq (Merkhan, 2014), whereas these values were similar to those reported for Turkish Awassi (Al-Samarai et al., 2015) raised at the Abu Garip Research Station located in Iraq. The daily milk yield obtained in the present study was lower than that reported in the improved Awassi sheep kept in intensive conditions (Pollott and Gootwine, 2001). Awassi sheep examined within the scope of the research reached the highest milk yield on day 75 of lactation (Figure 1), and the average lactation peak value was calculated to be 1639.12±80.76 g. This lactation peak value was between the values reported by Dağ and Zülkadir (2004) and that reported by Dağ (1996). In the present study, the total milk yield (82.92±5.46 kg) obtained until day 75, when the highest milk yield was observed, constituted 50% of the total milk yield (166.88±8.53 kg) obtained throughout the lactation period (Table 2). According to the results of this study, Awassi sheep reached approximately half of the lactation yield during the peak period of lactation and the remaining half was obtained in the last 90 days. In terms of additive milk yield, the ratio of peak milk yield to total milk yield varies between breeds (Esen and Özbey, 2002; Ünal et al., 2002; Yardımcı and Özbeyaz, 2001; Kahraman and Yüceer Özkul, 2020).

The first and second lactation data of Awassi sheep in the present study had an average value compared to that reported in literature. Milk yield is characterized by various factors (Akçapınar, 2000; Akçapınar and Özbeyaz, 1999; Kaymakçı 2010). Part of the difference in milk yield is due to environmental differences. On the other hand, when milk yield in Awassi sheep was examined, a wide variation within the breed was observed. Using this variation, elite herds with high milk production were obtained through breeding studies conducted in Awassi sheep in different parts of the world. Following these selection studies, a good level of genetic improvement was achieved in milk

yield, and dairy herds were formed within the breed (Pollott and Gootwine, 2001). Therefore, it is thought that the genetic and biochemical factors as well as the environmental effects contribute to the difference in milk yield in Awassi sheep. Indeed, the average lactation milk yield in low- and high-yielding sheep was found to be 36.96 and 297.15 kg, respectively in the present study (Table 3). It can be concluded that the difference in yield observed between these groups, which have similar environmental conditions, mode of delivery, and milking type, is due to the genetic effect in favor of increased milk yield. This variation among the Awassi breeds allows rapid genetic improvement within the breed to develop high-yielding herds. High-yielding Awassi sheep can be used as a parent line to raise dairy breed sheep.

Milk fat ratio, one of the quality parameters examined in the present study, inversely increased with milk yield from early lactation, and the maximum fat rate value was observed on day 135 of lactation. This change observed in milk fat ratio during lactation was consistent with that reported in some indigenous and crossbred sheep (Kahraman and Yüceer Özkul, 2020) and Akkaraman and Awassi breeds (Aktaş, 1970). The total average fat content during lactation for Awassi sheep in the present study was lower than that reported for Awassi and Akkaraman sheep (Yalçın and Aktaş, 1969) and for Awassi sheep reported by Gürsu and Aygün (2014). The values of fat ratio in the present study were similar to those reported for Awassi sheep by Aktaş (1970); however, they were similar or higher than those reported for indigenous and crossbred sheep by Kahraman and Yüceer Özkul (2020).

During the lactation period, the protein ratio in milk tends to increase from early lactation, and the highest protein ratio was achieved on day 105 of lactation. The total average protein ratio was similar to that reported by Özder (2002) for Türkgeldi sheep and lower than that value for Awassi breed by Konar et al. (1991) and Şahan et al. (2005).

The milk lactose values of Awassi sheep in the present study were similar to those reported for Akkaraman sheep by Kahraman and Yüceer Özkul (2020) and higher than those reported in Awassi sheep by Konar et al. (1991).

Dry matter ratio increased from the start of lactation to day 105 in the present study. It was considered that the increase in dry matter observed during this lactation period was due to the increase in fat and protein ratios and decline in the amount of milk during the same period. Indeed, many studies have reported high level of positive correlation between dry matter and fat and protein ratios (Çelik and Özdemir, 2003; Kahraman and Yüceer Özkul, 2020). The total average dry matter ratio determined for sheep milk in the present study was lower than that reported in a study that examined the chemical change during lactation in Awassi sheep milk (Şahan et al., 2005) as well as that reported for Awassi sheep by Gürsu and Aygün (2014). The results of the present study were consistent with those reported for Tuj sheep (Karaoğlu et al., 2001); however, they were lower than those reported by Özyürek (2020). There are different reports on sheep milk composition in literature. These differences are due to genetic and environmental factors such as breed, milking processes, feeding, breeding, and climatic conditions (Akçapınar and Özbeyaz, 1999; Çelik and Özdemir, 2003; Pugliese et al., 2000). The differences in the device and method used in the analysis of milk content should not be ignored while evaluating these results.

SCC decreased from the start of lactation to the middle period of the study and tended to rapidly increase during the last period. This change in SCC was similar to that reported in literature (Pirisi et al., 2000; Kahraman and Yüceer Özkul, 2020). SCC calculated throughout the lactation period in the present study was within the range reported for healthy

sheep milk (Yağcı and Kaymaz, 2006). During lactation, the pH value showed a steady decreasing trend, which was in accordance with the continuous decrease in Awassi sheep (Şahan et al., 2005) reported in a previous study.

In the present study, the L* and a* values of 72.69±0.16 and −6.11±0.04, respectively, were lower than those reported by Priolo et al. (2003), Doğan and Boztepe (2012), and Yüceer et al. (2015). The b* value in the present study was higher than that reported by Priolo et al. (2003) and Yüceer et al. (2015), whereas it was similar to that reported by Doğan and Boztepe (2012). The differences in color data of sheep milk are due to several genetic and nongenetic variables, such as feeding, disease, sampling, and lactation period (Doğan and Boztepe, 2012).

The amount of butyric acid (C4:0), which is one of the short-chain fatty acids, was higher in all groups in the present study than that previously reported for sheep milk. Milk and dairy products obtained from Awassi sheep are fondly consumed with their unique taste and flavor, especially in XXXX. Therefore, this flavor plays an important role in the choice of milk and dairy products by the local people. Butyric acid (C4:0), which involved in the formation of the characteristic flavor of sheep milk (Park et al. 2007), found in different amounts in Awassi sheep milk, together with other proportionally different fatty acids, has an important effect on breed-specific milk flavor. However, the high amount of short- and medium-chain fatty acids (C4:0 and C6:0, C8:0 and C10:0) in the milk of Awassi sheep was considered to have negative effect on the milk quality. Previous studies on the comparison of fatty acids in sheep milk have reported different results. This is due to the fact that the composition of fatty acids is affected by the rations used in animal nutrition, genotypic differences, and ration × breed interactions (Chilliard and Ferlay, 2004; Tsiplakou and Zervas, 2013).

CONCLUSION

There is a wide variation in milk yield within Awassi sheep breed. These variations may allow rapid genetic improvement to establish dairy elite herds of Awassi breed. The values of milk quality parameters were in range of those reported for sheep milk.

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Conflict of interest: The authors declare that they have no conflict of interest.

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BVDV monitoring by pooling and real time RT-PCR as economical monitoring technique with low BVDV prevalence

ABSTRACT

The aim of this study was to determine whether the use of pooled blood samples and real-time RT-PCR are feasible for detecting BVDV in infected animals. For this purpose, blood samples obtained from 2701 cattle, brought from 62 different farms, were used to test for the presence of bovine viral diarrhoea virus (BVDV) at the Veterinary Virology Department of Aksaray University Faculty of Veterinary Medicine. The sampling was done from all geographical regions in Turkey. Blood samples were pooled in groups of eight, creating a total of 342 pools. Each pool was checked for BVDV with a real-time RT-PCR test. BVDV nucleic acid was detected in 18 (5.26%) of a total of 342 pools. BVDV was detected in 18 of 62 farms. The most important result obtained from this study is that BVDV monitoring by pooling and real time RT-PCR can be done very economically when the disease prevalence is low (<10%). A risk/benefit estimation can be done for breeders who want to start vaccination programs.

Keywords: BVDV, Pooling, Real Time RT-PCR

INTRODUCTION

Bovine viral diarrhoea virus (BVDV) infection was first described by researchers at Cornell University in the USA (Olafson et al., 1946). This infection is an important disease that affects the reproductive system in cattle all over the world (Grooms, 2004). The resulting infection creates clinical signs characterized by diarrhoea, thrombocytopenia, hemorrhage, respiratory tract diseases, gastrointestinal system ulcers, abortion, reproductive disorders, and growth retardation (McGowan et al., 1993; Blanchard et al., 2010). Infection-related reproductive disorders have an important economic impact on cattle breeding (Houe, 2003; Grooms, 2004; Richter et al., 2017). The resulting economic losses are mostly related to death and weight loss caused by the infection of the digestive system, and reduced reproductive performance as a result of a transplacental infection in the reproductive system. Births of persistently infected (PI) calves that spread the virus for life are important in transmission (Ames, 1986; Baker, 1995; Bowen, 2011; OIE, 2017). Therefore, the disease is one of the most important threats to sustainable cattle breeding (Gunn et al., 2005).

BVDV, which is the causative agent, is a prototype virus in the pestivirus genus in the Flaviviridae family (Bowen, 2011; Koonin et al., 2020).

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Research Article

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The agent, which is serologically uniform, exhibits two biological (biotype) different characters (cytopathogen-cp and non-cytopathogen-ncp). It has been reported that the relationship between biotypes plays a decisive role in the course and pathogenesis of infection (Brownlie, 1991). BVDV infection has been a common disease for many years all over the world (Ridpath, 2010) and observed in Turkey for many years (Öncül et al., 1964; Alkan and Burgu, 1993; Çabalar and Karaoğlu, 1999; Duman et al., 2009; Okur et al., 2007; Tan et al., 2006; Yılmaz et al., 2012; Yeşilbağ et al., 2014; Yılmaz, 2016).

One of the most important results of BVDV infection is the presence of PI calves. PI calves are formed as a result of infected cows with noncytopathogenic virus in the first 3 months of fetal life (Stokstad and Loken, 2002). These calves born as virus carriers are a source of contamination throughout their lives. PI animals have an important role in the presence and persistence of BVDV infection in cattle populations (Moerman et al., 1993). Reported PI BVDV infection in cattle populations around the world is up to 2.00% (Brock, 2003; Peterhans et al., 2003; Smith et al., 2008; Stahl and Alenius, 2012; Newcomer et al., 2015; OIE, 2017). In Turkey, this ratio is not homogeneous, and it is reported to vary up to 6.50% (Şimşek, 1997; Burgu et al., 2003; Tan et al., 2006; Yeşilbağ et al., 2012; Yılmaz et al., 2012; Avcı and Yavru, 2013; Yavru et al., 2013; Şimşek et al., 2017).

It has been reported that persistent infected (PI) animals are responsible for the spread of the disease and have a role in direct and indirect transmission by spreading viruses throughout their lives (Houe, 1999). Therefore, early diagnosis and elimination of these PI animals in the population is important for disease control. To control and eradicate this disease, mostly "vaccination, systematic control, removal of PI animals, movement controls of infected herds, strict biosecurity, and surveillance" methods are recommended (Lindberg, 2003; Rypuła et al., 2013; OIE, 2017; Moening ve Becher, 2018). In recent years, it has become important to eliminate newborns with positive BVDV from the herd (Nelson et al., 2015). Also, foetal protection of newborns is very important to control the disease in herds.There are several publications about some vaccines providing fetal protection against BVDV infection (Bolin 1995, Brownlie et al., 2000, Patel et al., 2002, Dubovi 1992, McArthur, 2004).

Direct and indirect virological test methods are used in the diagnosis of the disease in infected or suspicious herds. For this purpose, Enzyme Linked Immunosorbent Assay (ELISA) and molecular tests are widely recommended methods (Edwars, 1990; Neill et al., 2014; OIE, 2017). In recent years, real-time RT-PCR is one of the mostly used molecular tests.

For many years, a single animal sample has often been used in tests, often said to be the best practice. On the other hand, using pooled samples is an alternative option for economic reasons. Pooling strategy was previously used in diseases that affect people with high testing costs (Kline et al., 1989). Pooled testing offers a cost-effective advantage, especially in disease cases with low (<10%) prevalence (Dorfman, 1943; Cowling et al., 1999). The pooling protocols have been presented by different researchers as a general application to screening using a sensitive diagnostic test to detect diseases or pathogens in populations for many years (Boulard and Villejoubert, 1991; Rodake et al., 1997; Munoz-Zanzi et al., 2000; Lanyon et al., 2014; Furstenau et al., 2020).

In this study, the aim is to determine whether the use of pooled samples and real-time RT-PCR are feasible for detecting BVDV in viremic or PI animals. The economic comparison was made according to the cost of commercially available individual AgELISA herd screenings. It is the first study to use

pooled samples and real-time RT-PCR together for BVDV diagnosis in Turkey.

MATERIAL and METHOD

Blood samples

Within the scope of the study, no extra sample collection was performed as blood samples that were brought to the Laboratory of Veterinary Virology Department in Aksaray University Faculty of Veterinary Medicine by dairy farms for diagnosis of BVDV disease were used. A total of 2701 EDTA blood samples from 62 different dairy farms with relatively good management practice in terms of biosecurity, located in seven regions of Turkey (Aegean, Black Sea, Central Anatolia, East Anatolia, Marmara, Mediterranean, South-East Anatolia) were tested. EDTA blood samples were combined in groups of maximum eight as a pool. The calculaton of pooling size is based on a publication (Munoz-Zanzi et al., 2000). This group calculated that 8 samples were economical for flocks with a prevalence of 2 percent (Munoz-Zanzi et al., 2000). 342 pools in total were created. The EDTA blood samples used in the study were stored at -20°C until they were analyzed without any pre-treatment.

Kits

RNA isolation from the pooled blood samples was performed using the Real PCR DNA/RNA Spin Column Kit (IDEXX, Montpellier, France) and a real-time RT-PCR test was performed using Real PCR BVDV RNA Test (IDEXX, Montpellier, France). Kits were used according to the manufacturer's instructions.

The test contains specific primers and probes designed for BVDV-1, BVDV-2, BVDV-3 and Border Disease Virus.

Controls

PCR positive control and BVDV positive blood samples in the stock of the Laboratory of Veterinary Virology Department in Aksaray University Faculty of Veterinary Medicine were used as positive controls. Distilled water and PCR negative control were used as negative controls. The internal controls (PCR positive control, PCR negative control, and internal controls) were included in the kit (IDEXX RealPCR BVDV RNA Test, IDEXX, Montpellier, France).

Statistical analysis

Statistical differences between pools, farms and regions for BVDV positivity were assessed through Pearson's Chi-square using SPSS v.22.0 software. The calculared "P" value less than 0.05 was regarded as statistically significant.

RESULTS

A total of 2701 EDTA blood samples of different ages, breeds, and sex were pooled in groups of maximum eight individual samples. As a result of the test, pestivirus nucleic acids were detected in 18 pools (5.26%). The 18 pools consisted of a total of 132 individual blood samples. Unfortunately, it was not possible to test those 132 blood samples individually. Therefore, the positive results detected were thought to indicate a possible BVDV infection (viremic or PI). If all are considered positive, this corresponds to 4.89% prevalence (Table 1). Eleven out of 62 (17,74%) farms were detected as positive for pestivirus antigens (Table 1).

Table 1. The distribution and test results of the samples used in the study

Geographical Regions of Turkey	Number of			Pestivirus Negative			Pestivirus Positive		
	Blood Samples	Pools	Farms	Blood Samples	Pools	Farms	Blood Samples	Pools	Farms
Mediterranean	237	30	9	221	28	7	16	2	2
East Anatolia	106	14	3	90	12	1	16	2	2
Aegean	381	48	7	365	46	5	16	2	2
South-East Anatolia	80	10	1	80	10	1	0	0	0
Central Anatolia	922	116	24	904	113	22	18	3	2
Black Sea	98	13	2	98	13	2	0	0	0
Marmara	877	111	16	811	102	13	66	9	3
Total Number and (%)*	**2701 (100)**	**342 (100)**	**62 (100)**	**2569 (95,11)**	**324 (94,74)***	**51 (82,26)**	**132 (4,89)**	**18 (5,26)***	**11 (17,74)**

*There was no significant differences between the regions where Pestivirus were detected in pools (P = 0.297).

DISCUSSION

BVDV infection has been detected in cattle in all countries where prevalence studies have been conducted (Lindberg, 2003). This situation is an indication that the virus is widespread in the world, and also expresses the spread potential of the agent with international live animal trade.

The control of BVDV infection is usually done by combining test and elimination, preventive vaccination, and strict biosecurity practices (Lindberg, 2003; Rypuła et al., 2013; OIE, 2017; Moening and Becher, 2018). With these methods, successful eradication programs are implemented in Europe (Switzerland, Germany, Austria, Luxemburg, Ireland) (Hanon et al., 2017). In these countries, breeders participate in the control-eradication programs on a mandatory or voluntary basis. According to the accessible literature data, there are no similar national programs in Turkey. However, conscious breeders apply similar practices in their own farms. The high cost of testing is shown as the most important reason why the majority keep their distance from this issue. In this respect, the use of pooled samples in tests should be considered as a very good alternative in order to reduce test costs and apply a sustainable combat strategy. Pooling strategy was first proposed to improve the efficiency of large-scale pathogen screening campaigns by Robert Dorfman (1943) during the second world war. It was previously used in diseases that affect people with high testing costs (Kline et al., 1989). Pooled testing offers a cost-effective advantage, especially in disease cases with low (<10%) prevalence (Dorfman, 1943; Cowling et al., 1999). The pooling protocols have been described by different researchers as a general application to screening using a sensitive diagnostic test to detect diseases or pathogens in populations for many years (Boulard and Villejoubert, 1991; Rodake et al., 1997; Munoz-Zanzi et al., 2000; Lanyon et al., 2014). It is widely used in disease-free or low prevalence herds in the USA (Kennedy et al., 2006). An evaluation in terms of current test prices can be made with the analysis fees announced by the laboratories affiliated to the Ministry of Agriculture and Forestry (URL 1). The AgELISA test fee has been announced as approximately 158 TL/sample and the real time RT-PCR test fee is approximately 454 TL/sample by the Ministry. Considering 8 sample pooling, the real time RT-PCR test fee can be calculated as approximately 56 TL/sample. The cost of per sample would be quite low even if retests were to be included. As can be seen, the proposed method with this study seems to be quite advantageous in herd-based BVDV control and eradication studies.

The results presented herein describe the prevalence of BVDV in EDTA blood sample pools tested by commercially available real-time RT-PCR. The test is ideal for use in low-prevalence situations using pooled samples as the detection limit of RT-PCR is very low. Considering the results of previous studies on

this subject in Turkey, the country can be considered as a country with low prevalence (<10%) in terms of disease.

In this study, viremic or PI (5.26%) animals were detected in 18 out of 62 farms (17.74%), in other words, 18 out of 342 pools of 2701 blood samples. However, we expected a higher prevalence of BVDV in Turkey based on previous serological studies which reported in average seropositivity of 50% and more (Yılmaz, 2016; Timurkan ve Aydın, 2019). The results from this study indicate a low prevalence in the tested farms. In particular, sample pooling is a very economical alternative to the general rule of "continuous monitoring of herds for BVDV and eliminating positive animals". In this study, demonstrating that there was no positivity in the farms sampled from the Black Sea and South-East Anatolia regions significantly reduced the test cost and data was obtained for these farms to start the vaccination program as soon as possible. Polak et al. (2016) especially indicate; "vaccination will be effective only when it is carried out correctly and preferably after previous determination of BVDV infection status". In order to reach a similar result, farms in regions other than Marmara will be able to get situations with a few tests and start vaccination programs safely.

The pooling method has been described in a thesis in relation to BVDV in Turkey. 160 blood samples were pooled in pairs and it was emphasized that two (2.5%) out of 80 pools were positive with RT-PCR (Sarıkaya et al., 2012). In our study, an important economic advantage is revealed by pooling in groups of eight with real time RT-PCR. Mars and Van Maanen (2005) estimated the optimum pool size as 36, based on the costs of RT-PCR versus the costs of individual AgELISA's in relation with the probability that a pool is PCR-positive. This estimation is based on Dutch cattle farming conditions with low BVDV prevalences. This means that also under the Turkish conditions with low BVDV prevalences, the number of individuals per pool may still be increased (more then 8 per pool) to make this test even more economically viable. For the detection of PIs in the Belgian eradication program, blood samples are gathered into pools of 30 and tested by RT-PCR (Laureyns et al., 2010).

In this study, the fact that 17.74% of the tested farms were BVDV positive confirms the lack of continuous monitoring and BVDV control among the breeders. The value of the results obtained in terms of controlling BVDV should not be underestimated as infected individuals should be quickly removed from the herd. It has been reported that 85% of the herd is in contact with the infection within 2 years following the introduction of a PI in a herd that was not immunized against BVDV infection and a very high rate of persistent viremic calf births were observed in the herd (Moerman et al., 1993).

Disease control including vaccination is an important preventive practice, so that herd immunity is kept at the highest level. Vaccines providing foetal protection can also be used for this purpose. However, vaccination should be done correctly in herds. In herds to be vaccinated, it is recommended to first determine the immune status of the herds in terms of the agent to be vaccinated (Polak et al., 2016). It will be useful to identify and eliminate carrier individuals in the herd before vaccination with the methods used in this study.

CONCLUSION

To conclude, the results obtained in this study confirm the presence of BVDV in cattle herds in Turkey. Periodic monitoring of cattle farms for presence of BVDV, elimination of PI individuals, and vaccination are recommended. Considering the relatively low prevalence (<10%) for BVDV infection, professional support is strongly recommended to control

BVDV in Turkish herds. Pooled-sample testing by real time RT-PCR lends itself to screening herds for low prevalence agents at an economically viable way. This may be of interest in animal disease surveillance, and herd certification programs. It is necessary to pay attention to some issues when working with sample pooling. Recent vaccination of herds with modified live BVDV vaccine must be taken into attention. Also, sensitivity and specificity of PCR are so important.

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Ethical approval: An ethical approval is not needed according Turkish regulations as only samples were taken for diagnostic purposes.

Conflict of interest: There is no conflict of interest.

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Molecular and serological investigation of Bovine Viral Diarrhea Virus in dairy cattle in Konya province

Research Article

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ABSTRACT

Bovine viral diarrhea virus (BVDV) infection is a viral disease observed in many parts of the world and causing significant economic losses in dairy cattle enterprises. The present study was carried out to determine the seropositivity of BVDV and perform the molecular detection of the virus in cattle in dairy enterprises situated in Konya province and its surroundings in the Central Anatolia Region. In this cross-sectional study performed between March 2017 and April 2019, a total of 393 serum samples were collected from twenty-four dairy cattle enterprises by random sampling. The presence of antibodies in the obtained blood serum samples was investigated by the virus neutralization test using NADL, the BVDV reference strain. Samples were controlled for BVDV specific antibody (Ab) presence and titter values using serum neutralization test. The serum samples were examined in terms of the presence of BVDV-specific antigens and specific RNA using a commercial ELISA kit and the RT-PCR method, respectively. According to the results of the analysis, the animal and herd-level seropositivity was 55.72% (219/393) and 79.16% (19/24), respectively. It was revealed that seropositivity between age groups was statistically significant (χ2:11.81; p=0.002). Moreover, the samples were determined to be 45.13%, 60.53%, and 73.07% seropositive in the 6 months-2 years, 2-5 years, and above 5 years age ranges, respectively. It was revealed that all of the samples tested to detect persistently infected animals were negative for antigen and BVDV-specific RNA. As a result, it indicates the presence of BVDV infection in dairy cattle enterprises in Konya province. Therefore, it is essential for the country's economy to prevent the spread of the infection in question and implement voluntary eradication programs.

Keywords: Bovine viral diarrhoea virus, ELISA, RT-PCR, seropositivity, virus neutralization test, cattle

INTRODUCTION

Bovine viral diarrhea (BVD) is a viral disease that leads to poor reproductive performance, low milk yield, abortions, congenital anomalies, early embryo deaths, and births of persistently infected (PI) calves in adult cattle and causes considerable economic losses in cattle breeding worldwide (Handel et al., 2011; Ran et al., 2019; Şevik, 2021).

Bovine viral diarrhea virus (BVDV) belongs to the *pestivirus* subgroup of the *Flaviviridae* family. The viral genome is approximately 12.5 kb in size and has a large open reading frame (ORF) ending in the 5' and 3' non-coding end (Kokkonos et al., 2020). BVDV, which is in antigenic affinity with classical swine fever and border disease viruses, has two biotypes: cytopathic and non-cytopathic according to the proliferation status in cell cultures (Schweizer et al., 2006; Abe et al., 2016).

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The infection has three clinical forms: (a) acute transient form, (b) PI form, and (c) mucosal disease form characterized by severe lesions in the oral and intestinal mucosa, diarrhea, and death (Handel et al., 2011).

Fetal infections and births of PI calves are important in the epidemiology of BVDV. The causative agent is spread with secretions and extracts such as nasal discharge, semen, stool, tear discharge, blood, and milk (Passler et al., 2007; Yavru et al., 2013). The virus can be transmitted vertically to the fetus in pregnant animals. The timing of transmission of BVDV infection is of critical importance to the epidemiology of the disease. Infection occurring in the first 3 months of pregnancy may result in fetal death or the birth of a live PI calf permanently infected with BVDV due to the non-development of fetal immune competence. PI animals play the role of the most important source of transmission of BVDV since they carry the causative agent throughout their lives and spread it with their whole-body secretions (Houe, 1999; Scharnböck et al., 2018).

The complex pathogenesis of the disease caused by BVDV and sometimes the insidious nature of infections caused by BVDV in cattle represent a significant challenge for diagnosis (Horner et al., 1995). The virus neutralization test (VNT) is among the tests with high sensitivity and specificity in the detection of BVDV and is accepted as the gold standard (Kampa, 2006). Nowadays, ELISA tests are also employed to diagnose BVDV due to their advantages, such as obtaining faster results (Yavru et al., 2013). Moreover, it has become possible to detect the nucleic acids of the virus and diagnose the disease in a short time owing to technological developments (OIE, 2018).

For a voluntary BVDV eradication project, which is likely to be established on a national basis in the future, information on the epidemiological course of the disease on a regional basis is required to set priorities and raise the awareness of breeders. Thus, this study aimed to investigate BVDV molecularly and serologically in dairy cattle enterprises in Konya province and its surroundings in the Central Anatolia Region.

MATERIAL and METHOD

Sample collection and study design

This cross-sectional study was carried out on 393 animals aged six months and above in twenty-four dairy cattle enterprises in Konya province and its surroundings located in the Central Anatolia Region between 36°41' and 39°16' north latitudes and 31°14' and 34°26' east longitudes between March 2017-April 2019. In the sampled family enterprises with the number of animals in the range of 5 to 50 animals, the enterprise owners declared that cattle were not vaccinated against BVDV. The minimum sample size required was calculated as a total of 393 animals based on a mean expected BVDV prevalence of 50%, a confidence interval of 90%, a desired relative precision of 5%, and a design effect value of 1.45 by taking into account previously determined prevalence rates (McDermott and Schukken, 1994; Yavru et al., 2005, Avcı and Yavru, 2013; Dean et al., 2013). 10 ml of blood was drawn from the *Vena jugularis* of animals in accordance with animal welfare. Blood was centrifuged at 2500 rpm for 10 minutes to obtain serum. After blood serum were inactivated by being kept at 56 ^{0}C for 30 min, they were stored in a deep freezer at -20 ^{0}C until the test time. As part of the routine veterinary surgeon, the bloodletting process was performed with the consent of the animal owners to blood sampling and under the international recommendations (NRC, 2011; ADSA, 2020).

Laboratory analysis

Cell culture and test virus

Virus titration and VNT were performed with Madin Darby Bovine Kidney (MDBK) cells (ATCC CCL-22) and NADL strain of BVDV. Cells were cultured in Eagle's minimal essential medium supplemented with 2% penicillin/ streptomycin (Sigma-Aldrich, MO, USA) and 10% fetal calf serum. To apply the VNT test, tissue culture infective dose 50% ($TCID_{50}$) of the NADL strain was calculated by Spearman and Karber method (Hierholzer and Killington, 1996), and titer of strain was estimated as $10^{4.5}$ $TCID_{50}$/0.1ml. All positive serum samples were subsequently diluted in rates of 1/5, 1/10, 1/20, 1/40, 1/80, 1/160, 1/320, and tested again for the determination of titter values. As a result of the test, 1/5 above dilutions were accepted as positive.

Virus neutralization test

VNT is a highly sensitive and reliable method for the detection of cytopathogenic viruses, in the controls of specific antibodies. To detect neutralizing antibodies specific to the BVDV, the micro-virus neutralization test was applied according to the method described by Frey and Lies (1971). First of all, serum samples (50 µl) were twofold diluted in 96-well plates and then 100 $TCID_{50}$ of the NADL strain (50 µl) was added to each well. Virus and serum mixtures were incubated in incubator (Heal Force, Lishen, Shanghai Lishen Scientific Equipment Co. Ltd., Chin. Model HF151UV) at 37 °C with 5% CO_2 for 2 hr. Following incubation period, 50 µl MDBK cell suspensions (300,000 cell/ml) were added to each well, and plates were incubated for 3-5 days. Results were evaluated based on the absence or presence of cytopathological changes in cells.

Test results were evaluated on the basis of micromorphology of cells using inverted microscope (Olympus IX71, Japan. Model IX71 S8F-3). Later on, all Ab positive serum were diluted in a series as 1/5, 1/10….1/320 for BVDV and test applied to determine Ab titer values (50% serum neutralization test (SN_{50})).

Results of the SN_{50} were calculated according to the method described by Reed and Muench (Lorenz and Bögel, 1973). The virus used in present study are in cytopathogenic nature, thus, inhibition of virus growth indicated by non-destructed monolayers of cell cultures was evaluated as indicator of virus neutralization. For scoring a sample as positive for the investigated antibodies, both wells used for the same sample were asked to be free of cytopathogenic effect.

Antigen ELISA

Serum samples were examined for the presence of BVDV antigen using a commercial antigen kit (IDEXX BVDV Ag/Serum Plus IDEXX Laboratories, Westbrook, Maine, USA). Analysis was done on serum samples according to the instructions provided by the manufacturer. Ag ELISA results were calculated for each sample

$$S\text{-}N = ODS_{450} - ODN_{450controlmean}$$

where OD450: optical density at 450 nm of the sample (S) and negative control mean (N). The samples with the values of 0.30 and below were evaluated as negative and above 0.30 as positive

RNA extraction and one-step RT-PCR

Viral RNA in the serum samples of animals was obtained using a commercial extraction kit (RNeasy Mini Kit, Qiagen, Germany). Extraction was performed following the manufacturer's instructions. One-step RT-PCR analysis was conducted using the primers shown in Table 1. In the analysis, nuclease-free water was used as a negative control, and the RNA of the BVDV NADL strain obtained from Selçuk University, Faculty of Veterinary Medicine, Department of Virology was used as a positive control. One-step RT-PCR reaction

was performed using a commercial kit (OneStep RT-PCR Kit, Qiagen, Germany). To this end, a 25 µl reaction mix was prepared, containing 5 µl of 5 x RT-PCR buffer, 1 µL of the enzyme, 1 µM of each primer, and 2.5 µL of the sample RNA. Amplification was performed in a Techne Prime thermal cycler (BIO-RAD Model, T100 Thermal Cycler, Singapure) under the following conditions: 30 minutes at 50 °C and 15 minutes at 95 °C (reverse transcription), followed by 40 cycles including 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min with a final extension of 10 min at 72 °C (Vilcek et al., 1994). PCR products were examined by electrophoresis on a 1.5% agarose gel stained with Gelred (Biotium, USA) at 90 Volts for 1 hour.

Table 1: Details of primers used to detect BVDV by One step RT-PCR

Primer	Primer sequence	Amplikon size	Reference
324F	5' ATGCCCTTAGTAGGACTAGCA 3'	288 bp	Vilcek et al. (1994)
326R	5' TCAACTCCATGTGCCATGTAC 3'		

Statistical analysis

In the present study, the statistical analysis of the data was conducted using the R program (V: 3.6.0). Chi-squared tests were employed in the analysis of differences in the age groups. A value of $p<0.05$ was considered statistically significant.

RESULTS

Serological findings

The serum neutralization test applied to the 393 cattle blood serum samples were found to be positive for pestivirus specific antibodies in 219 (55.72%) of them. At the herd level, the presence of pestivirus antibodies was detected in 19 (79.16%) of 24 dairy cattle enterprises.

As a result of the microneutralization test applied to two-fold dilutions of blood serums in which the presence of BVDV-specific antibodies was detected, it was revealed that the BVDV antibody titer had antibodies in titers ranging from 1:5 to 1:160 and the peak value was 1:5. SN_{50} values were found as 1/5 in 29.68% of the total 219 serum samples found seropositive, 1/10 in 27.85%, 1/20 in 21.92%, 1/40 in 8.22%, 1/80 in 6.85%, and 1/160 in 5.48% (Figure 1).

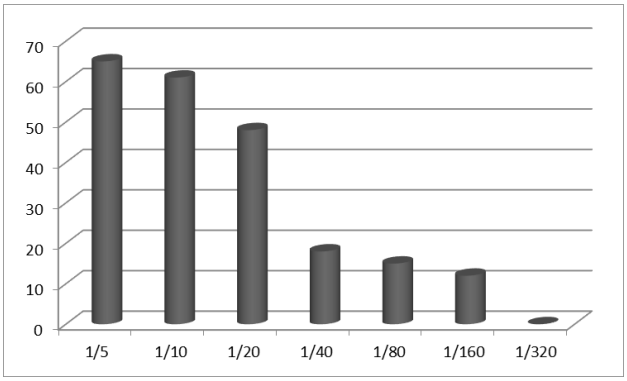

Figure 1: Ab titer distribution of BVDV seropositive samples (%)

Seropositivity was 45.13%, 60.53%, and 73.07% in the age ranges of 6 months-2 years, 2-5 years, and above 5 years, respectively. A statistically significant difference was revealed when the seropositivity rates in animals were compared based on age ($p<0.05$). (Table 2).

Antigen and BVDV-specific RNA detection

BVDV-specific RNA and antigen could not be detected in the 393 serum samples analyzed.

Table 2: Distribution of antibodies against BVDV in age groups of the tested animals

Age	No of tested	Pozitive	Negative	%
06 months -2 years	144	65	79	45.13
2-5 years	223	135	88	60.53
>5 years	26	19	7	73.07
Total	393	219	174	55.72
($\chi 2$:11.81, p=0.002)				

DISCUSSION

BVDV infection is among the viral infections that are quite common in Turkey, as in many countries of the world, and cause economic losses. The infection is usually subclinical in adult animals and is among the most important problems of cattle breeding as a herd problem due to its latent and persistent characteristics. In a study conducted using a survey and literature review on the prevalence of BVDV and covering the years 1960-2017, 88 countries confirmed the infection, and 107 countries reported infection reduction activities (Richter et al., 2019). The results of studies carried out worldwide in Brazil (48.8%) (Almeida et al., 2013), Bangladesh (51.1%) (Uddin et al., 2017), and Iran (54.3%) (Ghaemmaghami et al., 2013) and the results of this study are similar. However, studies performed in Uruguay (69%) (Guarino et al., 2008), Mexico (78.8%) (Milián-Suazo et al., 2016), and Ethiopia (69.8%) (Aragaw et al., 2018) revealed a high rate of seroprevalence.

In summary, BVDV seroprevalence studies carried out in various provinces and regions of Turkey reported seropositivity of 40.83% (İnce 2020), 74.9% (Gür, 2018), and 84.6% (Erol et al., 2014) in Afyonkarahisar, 68.51% in Antalya province and its surroundings (Demirsoy and Mamak, 2020, 86% in Aydın (Tan et al., 2006), 75.22% in Burdur (Bilgili and Mamak, 2019), 44.09% (Yavru et al., 2005), 46.22% (Avcı and Yavru, 2013), and 79.5% (Şimşek and Öztürk, 1997) in Konya and its surroundings, 20.19% in the provinces in the Black Sea region (Yazıcı et al., 2007), 96.8% in the Eastern and Southeastern regions (Çabalar and Karaoğlu, 1999), and 81.62% in the northeastern Anatolia region (Yıldırım and Burgu, 2005). The prevalence of BVDV infection in this study was determined as 55.72%. This rate is consistent with the rate obtained by Yavru et al., (2005), Avcı and Yavru (2013) in their study performed in Konya province and its surroundings. Upon evaluating these seropositivity rates, it is observed that BVDV infection is observed on the basis of provinces and regions with variable rates of prevalence in Turkey and in the world, and even studies conducted in the same region or province have determined different positivity values. These heterogeneities in seroprevalence studies in Turkey and in the world may originate from the different test methods used in the study, different regions of the country, time, and sample design.

In this study, in the distribution of the seropositivity of BVDV infection by age groups, 65 (45.33%) of the 144 animals in the age group of 6 months-2 years were seropositive and 79 (54.67%) animals were seronegative, 135 (60.53%) of the 223 animals in the age group of 2-5 years were seropositive and 88 (39.47%) were seronegative, and 19

(73.07%) of the 26 animals in the age group of 5 years and above were seropositive and 7 (26.93%) were seronegative. The lowest seropositivity rate among the age groups was 45.33% in the age group of 6 months-2 years, while the highest seropositivity was 73.07% in the group with the age of 6 years and above. The difference between the age groups was statistically significant ($p<0.05$). Moreover, the relationship between age and seropositivity and seronegativity indicated that seronegativity decreased as age increased. As the age increases, the disease may be observed more commonly in elderly animals, depending on the increase in the rate of infection in animals and the immunological status. The results of our study are consistent with the high incidence of infection in adult animals, as reported in other studies (Mockeliuniene et al., 2004; Bilgili and Mamak, 2019; Erfani et al., 2019; Hou et al., 2019).

Additionally, a high rate of seropositivity in a herd is regarded as an indicator of the presence of PI animals in that herd, and the prevalence of PI animals in the herd varies by 1-2% worldwide (Houe, 1999). The PI rate for BVDV infection in Turkey varies between 0.07-4.9% (Avcı and Yavru, 2013). Burgu et al. (2003) found the PI rate as 0.61-0.83% in their study from the samples collected from dairy cattle enterprises in different regions of Turkey, and Yılmaz et al. (2012) determined the PI rate as 0.5% in blood samples collected from four different regions. Moreover, Şimşek and Öztürk (1997) reported the PI rate as 0.7% and Bulut et al. (2006) as 0.1% in Konya province. This study detected no PI animals. This suggests differences in the number of samples tested, the rate of retesting, and differences in the regions studied, or the low number of persistently infected animals in the herds sampled. Although PI animals could not be detected in the study, it should not be ignored that there may be economic losses due to BVDV in the region and throughout Turkey. It is a known fact that acute and PI animals ensure the circulation of the disease in the field. Although the number of PI animals is usually low, it ensures the main persistence of the virus in the herd. Therefore, it is important for future eradication programs to control animals in the enterprise periodically in terms of virology and determine whether newborns are PI.

Furthermore, studies on the molecular epidemiology of BVDV in Turkey may help monitor virus isolates circulating on a national scale. Detailed information about the molecular typing of BVDV provides useful information for the establishment of disease control programs (Yeşilbağ et al., 2008). In the study carried out by Şevik (2018) in Afyonkarahisar province, the samples collected from enterprises where abortion problems were detected (fetus tissue samples of sheep and cattle) were evaluated for the presence of BVDV nucleic acid using the real-time RT-PCR method, and 22.2% positivity was determined. Another study performed in Ankara, Çorum, Kırıkkale, and Yozgat provinces examined the presence of BVDV in cows with reported abortion and infertility problems, and 3.55% positivity was detected in the study carried out with RT-PCR in seropositive whole blood samples (Aslan et al., 2015). This study did not detect BVDV RNA in the samples tested using RT-PCR. The fact that the prevalence of PI animals with regard to BVDV infection in cattle populations in the world is generally low in the range of 0.5-2% (Houe, 1999) and the PI rate in Turkey is low in the range of 0.07-4.9% (Burgu et al., 2003; Avcı and Yavru, 2013) may partially explain the reason why BVDV nucleic acid could not be detected in serum samples in our study.

CONCLUSION

As a result, there is currently no control and eradication program for BVDV infection in Turkey. Establishing eradication programs within the framework of principles, including

identifying and eliminating persistently infected animals, increasing immunity against BVDV infection with vaccination, and implementing biosafety strategies, is a known reality already. Considering dairy cattle breeding throughout Turkey, it is thought that the share of small-medium-sized enterprises is important, and it will be beneficial in designing future eradication programs on this basis.

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Ethical approval: In this study, animal owners gave their consent to blood sampling at the stage of collecting samples and epidemiological data. Furthermore, blood sampling was carried out under the supervision of a veterinarian in accordance with international ethical standards (Directive 2010/63/UE).

Conflict of interest: None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper. The authors declare that they have no conflict of interest.

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The effects of humic acid addition to ration on the fattening performance and some oxidative stress parameters in Anatolian Merino lambs

Research Article

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ABSTRACT

In this study, it is aimed to determine the effects of adding humic acid to ration of Anatolian Merino lambs on the fattening performance and some oxidative stress level by using thiol / disulfide balance measurement method. In the study, singleton 32 male Anatolian Merino lambs with an average age of 3 months were used. The experiment was carried out by forming a control group without additives and totally four trial groups, three of which were added with humic acid (2, 4, 6 g/kg), with 8 replications in each group for a total of 70 days. The lambs housed in the group partitions were fed with 400 g/day/head of alfalfa grass as roughage and lamb grower feed ad-libitum containing 2750 kcal/kg DM ME, 16% CP until the end of the trial. At the end of the research; intra-group native thiol (NTL, µmol/l) values increased on the 30th day ($P<0.05$) in all groups except the control group, while total antioxidant status (TAS, mmol/l), total thiol (TTL, µmol/l) and disulfide values increased on the 60th day in all groups including the control group ($P\leq0.001$) was observed. In terms of lambs' feed consumption, live weights, body weight gains, feed conversion ratios, TAS, total oxidant status (TOS, µmol/l), oxidative stress index (OSI), TTL, NTL and disulfide was found that there was no significant difference between groups ($P>0.05$). It was concluded that the humic acid additive was not effective on the fattening performance, but 4 or 6 g/kg could be added to the lamb rations due to the increase in thiol groups, which have an important role in the antioxidant defense system.

Keywords: Humic acid, lamb, oxidative stress, ruminant, thiol/disulfide balance

INTRODUCTION

Various organic acids are used in livestock rations to increase the acidity of the feeds and prevent the deterioration of the feed, to maintain the balance between pathogens and beneficial microorganisms in the digestive system, to improve the digestion and absorption of ingested nutrients and to promote growth. Humic acid is one of these organic acids (Islam et al., 2005; Váradyová et al., 2009). These compounds originate from humus, which is formed by some substances such as carbohydrates, amino acids and phenols, which are released by the decay and decomposition of organic materials in the soil over time (Gau et al., 2001; Ying et al., 2001). They are defined as complex organic substances that are composed of humic, fulvic acid and some micro minerals, which can transfer electrons due to their chemical properties and can be chelate with many metal ions thanks to these properties (Ritchie and Perdue, 2003). In their natural state, they are insoluble in water and are not biologically active. The salts they form with the elements sodium, potassium and nitrogen are called "humate". Humates are soluble in water and biologically active (Eren et al., 2000; Küçükersan et al., 2005).

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The use of humic acids in animal feeds started with some humic acid preparations developed for the treatment of diarrhea and digestive disorders in calves, pigs, cats and dogs between 1980-1990. However, it is seen that humic acid products, which are known to be an important and effective substance in plant nutrition, are used to increase the efficiency of feed and drinking water of various animals (Demirulus, 2011).

The growth rate and yield potential of lambs are directly proportional to the feed efficiency level. Poor care and feeding conditions are effective in the deterioration of the oxidant-antioxidant balance of the organism and the formation of oxidative stress. This causes a decrease in the growth performance of the developing young ruminants (Altınçekiç, 2016; Serin, 2015). Oxidative stress can be defined as the deterioration of molecular and cellular functions as a result of the loss of the balance between the body's antioxidant defense and the production of free radicals that cause peroxidation of the lipid layer of the cells. Under oxidative stress, damage occurs to biomolecules such as lipids, proteins, and DNA. Free radicals; oxidized bases cause a variety of tissue damage, including DNA chain breaks and DNA-protein crosslink formation (Yokuş and Çakır, 2002). Oxidative damage caused by the rise of reactive oxygen species above physiological levels and the increase in the production of free radicals leads to damage to cell membrane lipids and weakening of cellular protein functions (Devasagayam et al., 2014; Pratic`o, 2005; Valko et al., 2004). Under normal conditions, there is a balance between free oxygen radicals and radical toxicity and the production of a protective antioxidant system. Oxidative stress, which occurs as a result of the disruption of this balance between antioxidants and oxidants in favor of oxidants, is a part of the mechanisms of cellular and molecular tissue damage in diseases (Celi, 2011). In the antioxidant defense system of the organism against free radicals, first of all, enzymatic or non-enzymatic antioxidant mechanisms in the cells come into play. Damage caused by radicals is prevented in the body by the enzyme systems of superoxide dismutase, catalase and glutathione S-transferase, as well as important biological thiols such as glutathione, cysteine, homocysteine, N-acetylcysteine, ɣ-glutamylcysteine. Thiols, also known as mercaptans, are organic chemical compounds containing hydrogen and sulfur atoms and sulfhydryl (-SH) groups attached to the carbon atom, which show antioxidant properties in preventing the formation of any oxidative stress state (Erel and Neşelioğlu, 2014; Sen and Packer, 2000; Turell et al., 2013). Thiols are involved in oxidation reactions via oxidants. They form covalent S-S bonds between the sulfhydryl groups of two cysteine amino acids in the structures of proteins. These are called disulfide bonds (Cremers and Jakob, 2013). Native thiols are molecules that contain an unreduced functional thiol group. They are responsible for the antioxidant defense mechanism. When oxidative stress increases, their amount decreases. Total thiol represents the thiol/disulfide level in equilibrium and total of oxidized-unoxidized thiols. Under oxidative stress conditions, lead to the formation of reversible disulfides bonds between oxidative residues of cysteine, low molecular thiol and protein thiol groups. The disulfide bonds formed can separate into thiol groups again. Thus, dynamic thiol/disulfide equilibrium can be achieved. It has a critical role in many cellular activities such as dynamic thiol/disulfide balance, antioxidant protection mechanism, enzymatic activity and cell growth. Today, it is a marker associated with many diseases in the medical field (Erel and Neşelioğlu, 2014). Thiol groups of sulfur-containing amino acids such as cysteine and methionine in proteins are the primary target point of reactive oxygen species. Oxidation of reactive oxygen species and thiol groups into

reversible disulfide bonds is the first manifestation of protein oxidation. Biological importance of thiols and disulfides; It can be explained by the preservation of the structure of proteins, the regulation of protein and enzyme functions, their roles in receptors, transporters and transcription (Ateş et al., 2015).

In this study, it was aimed to determine the effects of humic acid addition to the ration on fattening performance and some oxidative stress parameters by thiol/disulfide balance measurement method in Anatolian Merino lambs (TUIK, 2021), an important breed whose breeding is quite common in Turkey.

MATERIAL and METHOD

In the study average age of 3 months, singleton Anatolian Merino lambs were used as animal material. The research was carried out in May-June, when the average daily air temperature was 20-25°C. 32 Male lambs were selected from the Anatolian Merino herd in the farm, from male lambs with the same birth time and body weight as possible. The roughage material of the study consisted of dry alfalfa hay and the concentrate feed material consisted of lamb grower feed containing 2750 kcal/kg DM ME and 16% CP. A.O.A.C. (1984) was used as the method of determining the values of dry matter (DM) crude protein (CP), crude cellulose (CC), ether extract (EE) and crude ash (CA); Van Soest (1994) procedure was followed for determining the amounts neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). Metabolic energy (ME) (Anonymous, 2016), non-nitrogen extract (NNE=DM- (CP+CA+EE+CC)), cellulose (CL=ADF-ADL) and hemicellulose (HCL=NDF-ADF), values were derived from the analysis results on feed materials through calculation. The analysis results of the chemical compositions of the roughage and lamb grower feed used in the ration are given in Table 1.

Table 1. Chemical composition of feeds (%)

Nutritions	Lamb grower feed	Alfalfa hay
ME, kcal/kg DM	2750	1.38
DM	88.73	93.29
CP	16.00	9.17
CC	7.14	37.42
EE	2.89	0.99
CA	7.65	11.33
NNE	54.55	41.09
NDF	29.24	53.61
ADF	9.70	42.43
ADL	0.90	9.76
CL	8.8	32.67
HCL	19.54	11.18

The trial was carried out for a total of 70 days to reach the intended statistical data, the first 10 days of which was the adaptation to feed period, and the 60 days of the fattening period. At the beginning of the trial, the lambs were classified according to their first weighing body weights and divided into 4 groups homogeneous with 8 animals in each group randomly distributed. Group feeding was applied to the lambs. Health control of the lambs were also performed in the adaptation period. Trial groups were fed st group (no additive control group), 2^{nd} group (2 g/kg humic acid added), 3^{rd} group (4 g/kg humic acid added), 4^{th} group (6 g/kg humic acid added) with concentrated feed. While concentrated feeds were given ad-libitum, the excess feeds in the feeders were collected and weighed every

two weeks. Alfalfa hay used as roughage was given by weighing 400 g per animal per day, and at the end of two-week periods, the increased amounts in the manger were collected, weighed and recorded. Fresh and clean drinking water was always available in front of the lambs.

Body weights were determined individually by weighing every two weeks until the end of the trial, the first of which was at the beginning of the trial period. Weighings were made at the same hours (08:00) before the morning feeding. In each weighing period, the excess feed in front of the animals was collected and weighed, and the amount of feed consumed by removing from the amount of feed offered to the animals was recorded.

At the beginning (day 0), middle (day 30), and end (day 60) of the trial, blood samples were taken from the jugular veins into tubes with anticoagulants, from all lambs before the morning feeding. These blood samples were collected in flat gel tubes (Becton Dickinson and Company, New Jersey, USA), all samples were centrifuged at 3000 rpm for 10 minutes and stored at -80°C until analysis. Then, enzymatic and non-enzymatic measurements of all antioxidant and oxidant molecules were made in these blood samples with native thiol (--SH), total thiol (--SH+-- S--S--), Total antioxidant status (Total Antioxidant Level - TAS), Total oxidant status (Total Oxidant Level -TOS) kits (RelAssay Diagnostic, Turkey) (Erel and Neşelioğlu, 2014). The disulfide level was calculated with the formula (serum total thiol - serum native thiol)/2. All results are reported as micromoles per liter (µmol/l), TAS millimoles (mmol/l) (George and Hero, 1979).

For total thiol measurement, 10 µl of reagent 1 (R1) (10µl of R1' is used for free thiol measurement) and 10 µl of sample were mixed. Afterwards, R2 and R3 were added and the first absorbance (A1) reading was made spectrophotometrically at 415 nm wavelength (Schimadzu UV-1201 spectrofotometer, Kyoto, Japan). The second absorbance (A2) reading was taken at the same wavelength at the 10^{th} minute when the reaction peaked, and the measurement was completed by obtaining the A2-A1 absorbance difference. It was used 14.100 mol/l-1 cm-1 which is the molar extinction coefficient of 5-thiol-2-nitrobenzoic acid (TNB) for the calculation of total and free thiol levels.

Antioxidants in the sample convert the dark blue-green ABTS (3-ethyl-benzothiazoline 6 sulfonate) radical solution to the colorless ABTS form. The change in absorbance at 660 nm is related to the total amount of antioxidants. The kit has been calibrated with a stable antioxidant standard called Trolox Equivalent, similar to vitamin E. Oxidants in the sample oxidize the ferrous ion-clamp integrated with the ferric ion. The oxidation reaction is prolonged by the amplifying molecules present in the reaction medium. Ferric ion forms a colored compound with chromogen in acidic medium.

The total amount of oxidant molecules in the sample was determined in relation to the darkness of the color measured in the spectrophotometer. The kit was calibrated with hydrogen peroxide, the results were given as micromoles of hydrogen peroxide per liter (µmol H_2O_2 Equi v./l) (Erel and Neşelioğlu 2014). By taking the percentage of the ratio of TOS level to TAS level; OSI was calculated according to TOS (µmol H_2O_2 equiv/l) / TAS (mmol Trolox equiv/l) formula (Erel, 2005).

One-way analysis of variance (ANOVA) was used to test the significance of the difference between the independent group means in terms of each parameter studied, and the Duncan test was used to control the significance of the differences (Duncan, 1955; Düzgüneş et al., 1983). Data are given as arithmetic mean±standard deviation (X±SX).

RESULTS

The values of roughage, concentrate feed and total feed consumption averages obtained from lambs in two-week periods from the beginning of the study until the end of the trial are given in Table 2. The roughage consumption decreased in the 4th group, which consumed 6 g/kg humic acid added concentrate, in a 2-4 week period compared to the other groups (P<0.05). No statistically significant difference was observed between the 1st group, which is the control group without humic acid, and the other 3 groups with additives, in terms of roughage and concentrate feed and total feed consumption averages (P>0.05).

Table 2. Average values the consumption of roughage, concentrate and total feed of lambs

Properties	Trial Groups				P values *
	1	2	3	4	
Roughage, kg					
0-2 week	44.7±4.81	38.0±4.40	43.2±4.65	33.2±5.18	0.736
2-4 week	38.0±4.09[a]	24.8±3.87[ab]	34.8±3.75[a]	18.5±4.88[b]	<0.05
4-6 week	43.2±4.65	25.6±3.75	39.6±4.26	35.4±3.81	0.091
6-8 week	33.2±3.57	42.0±4.52	45.2±4.87	37.8±4.38	0.656
Concentrate, kg					
0-2 week	236±57.05	238±53.09	232±43.02	235±33.58	0.779
2-4 week	240±58.01	243±58.29	245±38.56	250±41.95	0.071
4-6 week	223±53.90	225±54.54	235±51.85	233±57.21	0.534
6-8 week	201±48.59	204±48.88	210±47.10	207±49.02	0.912
Total, kg					
0-2 week	280.7±32.36	276±30.78	275.2±26.39	268.2±22.16	0.753
2-4 week	271.8±29.56	267.8±23.42	279.8±24.43	268.5±25.14	0.193
4-6 week	261.4±51.75	250.6±22.61	274.6±23.28	268.4±19.94	0.261
6-8 week	247.0±23.07	246±22.20	255.2±19.93	244.8±19.35	0.853

*Difference between the averages shown with different lower case on the same line are significant.

In Table 3, the average values of the live weight (kg) and daily live weight gains (g) obtained in 2-week periods until the end of the trial, including the fattening initial body weights, are given. Accordingly, it was observed that 0, 2, 4 and 6 g/kg humic acid additives, respectively, did not differ statistically between the groups in terms of the specified parameters (P>0.05), and it was revealed that the humic acid additive did not affect the fattening performance at each dose.

Table 3. Average values of lambs live weight (kg), live weight gains (g/day)

Properties	Trial Groups				P values
	1	2	3	4	
Live weight, kg					
Beginning (0. day)	38.79±4.18	38.62±4.55	38.76±3.67	38.76±3.65	1.000
0-2 week	43.38±5.03	43.98±5.11	44.27±3.8	43.97±3.81	0.976
2-4 week	46.4±4.95	47.88±5.09	48.41±3.41	46.98±4.56	0.761
4-6 week	48.27±4.82	50.3±4.62	50.49±3.74	49.28±4.79	0.680
6-8 week	51.13±4.74	53.88±4.32	53.03±4.17	52.09±4.8	0.583
Live weight gain, kg/day					
0-2 week	4.59±2.31	5.36±1.01	5.51±0.6	5.21±3.0	0.752
2-4 week	3.02±0.77	3.9±0.79	4.14±0.8	3.01±4.34	0.593
4-6 week	1.87±0.92	2.42±1.58	2.08±0.58	2.3±0.72	0.639
6-8 week	2.86±0.47	3.58±0.95	2.54±0.88	2.81±0.89	0.057

As seen in Table 4, no statistically significant difference was observed between the group means in terms of TAS (mmol/l) values (P>0.05). On the other hand, when the in-group values of the 1st, 2nd and 3rd groups were examined, it was observed that the mean TAS (mmol/l) at the beginning of the experiment was found to be lower than the TAS (mmol/l) obtained on the 30th and 60th days (P<0.05). However, the mean of intergroup and intragroup differences in TOS (µmol/l) and OSI values were found to be insignificant (P>0.05).

Table 4. Averages of intergroup and intragroup TAS (mmol/l), TOS (µmol/l) and OSI values obtained during the trial

Properties	Trial Groups				P values
	1	**2**	**3**	**4**	
TAS					
0. day	1.17^{a}±0.21	1.09^{a}±0.10	1.13^{a}±0.11	1.65±1.04	0.113
30. day	1.54^{b}±0.11	1.40^{b}±0.16	1.46^{b}±0.22	1.49±0.15	0.363
60. day	1.59^{b}±0.19	1.59^{b}±0.12	1.53^{b}±0.14	1.61±0.16	0.636
*P values	**0.013**	**<0.001**	**0.004**	0.062	
TOS					
0. day	8.74±4.02	6.93±3.09	6.06±1.80	8.56±4.31	0.300
30. day	10.05±4.68	7.86±2.95	6.40±2.37	8.63±4.11	0.215
60. day	6.92±2.12	5.65±2.33	6.60±2.64	7.87±4.64	0.463
P values	0.459	0.121	0.368	0.895	
OSI					
0. day	0.74±0.31	0.65±0.33	0.55±0.18	0.65±0.40	0.647
30. day	0.65±0.29	0.55±0.17	0.43±0.15	0.57±0.24	0.227
60. day	0.44±0.13	0.36±0.15	0.43±0.15	0.47±0.25	0.532
P değerleri	0.264	0.059	0.236	0.717	

*Difference between the averages shown with different lower case on the same column are significant

The averages of the intragroup and intergroup differences of TTL (µmol/l), NTL (µmol/l) and disulfide values obtained from the groups during the trial are given in Table 5. It was observed that all three parameters did not differ in statistical significance between the groups (P>0.05). On the other hand, it was determined that the values of the mean TTL (µmol/l) values in each group were lower than the mean TTL (µmol/l) values obtained at the beginning of the trial (day 0) and at the 30th day at the end of the trial (day 60) (P≤0.001). In terms of NTL (µmol/l), the unadded control group was not affected, while the other groups were affected by the humic acid additive, and the values obtained on the 30th day showed a significant increase compared to the 0th day (P<0.05). In other words, it was observed that the native thiol level in the plasma increased on the 30th day with the addition of 2, 4 or 6 g/kg humic acid. However, at the end of the trial, although this value increased at the same level for each group, the differences observed in the groups on the 60th day were found to be statistically insignificant (P>0.05). When the disulfide values were examined, it was determined that the disulfide values increased significantly at the end of the trial in each group on the 0th, 30th and 60th days (P≤0.001).

Table 5. Intragroup and intergroup averages of TTL (µmol/l), NTL (µmol/l) and disulfide values obtained during the trial

Properties	Trial Groups				P values
	1	**2**	**3**	**4**	
TTL					
0. day	396.37[a]±95.82	361.66[a]±73.65	390.44[a]±91.50	333.76[a]±127.44	0.523
30. day	483.31[a]±98.46	487.08[a]±85.28	474.91[a]±70.22	495.12[a]±123.64	0.976
60. day	1545.60[b]±97.03	1519.20[b]±85.96	1513.80[b]±56.58	1587.50[b]±76.20	0.222
*P values	**0.001**	**0.001**	**0.001**	**<0.001**	
NTL					
0. day	292.50±115.60	229.37[a]±71.46	205.80[a]±60.04	216.06[a]±115.11	0.864
30. day	382.17±152.74	386.06[b]±117.81	370.39[b]±104.07	420.77[b]±144.20	0.173
60. day	331.17±61.41	307.23[ab]±92.32	308.50[ab]±81.96	346.75[ab]±104.87	0.696
*P values	0.097	**0.008**	**0.002**	**0.013**	
Disulfide					
0. day	51.93[a]±42.92	66.14[a]±43.55	92.32[a]±49.86	58.85[a]±37.01	0.239
30. day	50.57[a]±55.50	50.51[a]±27.67	52.26[a]±36.50	37.18[a]±29.43	0.830
60. day	607.22[b]±37.41	605.99[b]±63.01	602.65[b]±55.92	620.38[b]±55.49	0.889
*P values	**0.005**	**0.005**	**<0.001**	**<0.001**	

*Difference between the averages shown with different lower case on the same column are significant

DISCUSSION

In general, no research has been found in the literature on the evaluation of the oxidative stress level with the addition of humic acid to the ration by measuring the thiol/disulfide balance in ruminants. Therefore, the comparison of the results obtained is limited.

Studies on humic substances are mostly concentrated in poultry, and studies on the use of humic acids as productivity enhancers in ruminants are limited. Kara et al. (2012), humic acid added to quail feeds at a rate of 0.5%; reported that they significantly increased the live weight, live weight gain and feed conversion ratio. It has been reported that humic acid compounds provide optimum pH formation in the digestive tract, suppress harmful bacterial species, reduce mycotoxin levels and contribute to the development of intestinal health (Islam et al., 2005). It has also been emphasized that they significantly reduce digestive disorders and have antiviral and antibacterial effects (Taklimi et al. 2012). Studies on the mechanism of action of humic acid on fattening performance may give conflicting results on the subject. In this study, humic acid was not effective on fattening performance parameters of lambs. Although there are studies reporting that the fattening performance improves with the increase in feed efficiency and growth rate in lambs fed with humic acid supplementation (Covington, 2012; Wang et al., 2020), there are also studies reporting that humic acid compounds have no effect on performance, in support with the results obtained from this study (McMurphy et al., 2009; Silva et al., 2011). Fattening performance is under the influence of many factors such as breed, gender, age, care and feeding style, amount and quality of feed, and feed consumption increases in parallel with the age and live weight of lambs (Esen and Yıldız, 2000). In a study conducted by Şahin and Boztepe (2010) to determine the effects of live weight per fattening on fattening performance in Anatolian Merino male lambs, the difference between the groups in terms of live weight, daily average live weight gain and feed conversion ratio was found to be statistically insignificant. Consistent with the results of this study, the fact that the fattening performance parameters did not change may be related to the race. This result is also consistent with the results of a study showing that the humic acid contribution was not effective on the growth performance of kids (El-Zaiat et al., 2018).

Also, it was reported that humate administration at 10 ml/day and 15 ml/day for 8 weeks improved growth performance in the newborn kids and also had an effect at 21 d on skin reaction to phytohemagglutinin suggesting a possible effect on cell-mediated immune response (Agazzi et al., 2007). It has been emphasized that the addition of 1% and 2% humic acid in lambs diets increases the daily average weight gain without affecting the feed conversion rate, but 5% humic acid can reduce the growth performance of the lambs (Covington, 2012). On the other hand, there are studies reporting that the addition of humic acid does not affect blood biochemical parameters in sheep (Tunç and Yörük 2012), rams (Galip et al., 2010), beef cattles (McMurphy et al., 2009) and calves (Silva et al., 2011). In this study, it was observed that roughage consumption decreased in the 3rd group fed with 4 g/kg humic acid added ration compared to the 4th group fed with 6 g/kg additive in the 2-4 week period. This may be related to the feed preference of the lambs as other fattening performance parameters did not change during the 2-4 week period and the total trial period. It has been stated that the lambs consume by choosing the feed they need for nutrients, and their feed preferences vary according to many factors such as species, age, environmental conditions and physiological condition of the animal (Çavuşoğlu and Akyürek, 2018). On the other hand, the increase in plasma NTL level in group 4 in the same period can be explained as the activation of antioxidant mechanisms when feed consumption is directed towards concentrate feed.

In a study conducted with quails, it was reported that the use of humic acid in the ration at high doses such as 600 mg/kg caused a decrease in antioxidant levels, and 360-480 mg/kg doses had no effect on TAS (İpek et al., 2008). The increase in TAS in the control, 1st and 2nd groups on the 60th day is an indication that antioxidant molecules in the plasma increase and resistance against diseases develops. TAS reveals the total activity of all substances with antioxidant properties in the serum. It is expected that feeding conditions that require high energy for many body functions increase the level of oxidative stress (Koch and Hill, 2017). The fact that TOS and OSI did not differ in all other groups may be due to be healthy of the animals and the adequate energy content of the feeds. While TOS increases significantly in disease states, TAS and OSI tend to decrease (Mert et al., 2019). Although it is not statistically affected by humic acid supplementation, it can be said that TOS was higher in the control group on the 30th day compared to the supplemented groups, and especially 4 g/kg humic acid supplementation may have an effect on reducing the oxidant level.

TTL and disulfide levels increased in all groups, while NTL increased in groups with humic acid supplementation. This increase is more pronounced in lambs fed with 4 g/kg and 6 g/kg humic acid additives. It is emphasized that humic acid prevents the formation of free radicals and reduces stress factors by supporting the immune system (Huber and Parzefal, 2007; Paciolla et al., 1998). In a study that carried out to investigate the effect of dystocia that the type of birth does not occur within physiological limits and requires interventions from the outside, on total thiol and native thiol, significant reducing were found in total thiol and native thiol levels in kids in the dystocia group compared to the normal birth group (Akkuş, 2021). Therefore, this result can also be associated with the fact that lambs are healthy born and healthy animals.

According to the results of this study, the fact that there was no statistical difference between the groups in terms of some oxidative stress parameters is similar to the results of the research conducted by Avcı et al. (2013) on Merino sheep. By measuring the dynamic thiol-

disulfide balance, which plays a role in the development of many diseases, a lot of information can be obtained related to the health and nutritional status of the animal (Erel and Neşelioğlu, 2014). Since the increase in disulfide values in each group at the end of the trial was found to be significant in the control group and the humic acid additive did not make any difference between the groups, it can be said that the additive did not have an effect on the thiol/disulfide balance.

CONCLUSION

It was determined that there was no statistically significant difference between the groups in terms of feed consumption, live weight and live weight gain of the lambs, and it was concluded that the humic acid additive did not have any effect on the fattening performance.

At the end of the research; It was observed that the mean NTL values within the group increased on the 30th day in all groups except the control group, and the TAS, TTL and disulfide mean values on the 60th day in all groups including the control group. At the beginning (day 0), middle (day 30), and end (day 60) of the trial; intragroup differences were found to be significant in terms of TAS, NTL and disulfide. It has been concluded that 4 or 6 g/kg humic acid can be added to lamb rations due to the increase in thiol groups, which have an important role in the antioxidant defense system.

Today, no research has been found in the literature on the evaluation of oxidative stress level with thiol/disulfide balance measurement regarding the nutrition of ruminants. The number of studies conducted in ruminants with the addition of humic acid is also very limited. There is a need for new research on the subject.

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Investigation of Respiratory Syncytial Virus (RSV) and Parainfluenza-3 (PI-3) virus by histopathological and immunohistochemical Methods in sheep and goats

ABSTRACT

Respiratory Syncytial Virus (RSV) and Parainfluenza type 3 (PI3) virus cause serious respiratory system infections in sheep and goats. It was aimed to investigate the localization and distribution of sheep and goat RSV and PI-3 in lung tissue by histopathological and immunohistochemical methods. The study material consisted of 30 sheep and 24 goat lung paraffin blocks from Samsun and its surrounding regions, which came to Samsun Control and Veterinary Research Institute between 2016-2019. Histopathological changes characterized by bronchial and bronchiolar epithelial degeneration and desquamation, interalveolar septal thickness, epithelialization of the alveolar epithelial surface and inflammatory cells in the alveolar lumen. In addition, hyperplasia of peribronchial lymphoid tissue, hyaline membrane formation and syncytial cells in alveoli were observed less frequently. Althought, bronchiolitis obliterans were not detected in any of the cases. In immunohistochemical staining, RSV antigen was detected in 50% of sheep and 54% of goats, and PI-3 antigen was detected in 40% of sheep and 50% of goats. RSV and PI-3 antigenic distribution of sheep and goats in bronchial and bronchiolar epithelium and cells debris and interalveolar septum were statistically similar ($p>0.05$). PI-3 antigen in goats was detected more intensely than sheep ($p<0.05$) in alveolar macrophages statistically. It was concluded that the localization of RSV and PI-3 antigens in sheep and goat lung tissue was detected similar by this study. In addition, it was determined that RSV and PI-3 antigens were common in sheep and goats and it was thought that vaccination should be done for protection.

Keywords: RSV, PI-3, immunohistochemistry, sheep, goat

INTRODUCTION

Parainfluenza type-3 (PI-3) and Respiratory syncytial virus (RSV) are in the *Paramyxoviridae* family and many hosts also cause respiratory tract infections (Maidana et al., 2012). Most interesting in the biology of paramyxovirus infections is their potential for interspecies infection (Ellis, 2010). RSV is an enveloped, single-stranded, negative-susceptible RNA virus (Zaher et al., 2014) within the genus Pneumovirus (Van der Poel et al., 1995). RSVs have been identified as bovine, sheep, and goat RSV (Eleraky et al., 2001), and all RSV's have been reported to cause cross-species infections (Van der Poel et al., 1995). PI-3 is an enveloped, non-segmented, negative-sensitive RNA virus within the genus Respirovirus (Ellis, 2010; Newcomer et al., 2017). Bovine PI-3 has been reported to cause sheep and goat respiratory infections (Saeed et al., 2016; Stevenson and Hore, 1970).

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Research Article

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Additionally, viral agents such as bovine herpesvirus 1 (BHV-1), bovine viral diarrhea virus (BVDV), and bovine adenoviruses (BAV) cause respiratory tract infection in ruminant species (Al-Hammadi, 2016; Borujeni et al., 2020; Tamer et al., 2018) and cross-species infection between both small and large ruminant species (Yeşilbağ and Güngör, 2009).

RSV and PI-3 infection cause severe bronchopneumonia along with secondary bacterial infections in high-stress situations such as transport, air exchange, and nutrition (Haanes et al., 1997). The agent is transmitted by droplet infection and contaminated animal feed and water (Stott and Taylor, 1985). RSV targets both ciliary and non-ciliary bronchiolar epithelial cells and types II pneumocytes (Viuff et al., 1996), PI-3 virus, epithelial cells of the respiratory tract, and alveolar macrophages (Bryson et al., 1983). There are clinical signs in animals such as fever, weakness, nasal dishcarge, continuous and deep breathing, respiratory distress, cough, and loss of appetite. Histopathological findings are seen in the respiratory tract epithelium such as necrotic bronchiolitis, alveolitis, bronchi, bronchiole and type II pneumocyte hyperplasia, interalveolar septum thickening, atelectasis and lymphoid hyperplasia (Narita et al., 2002; Sacco et al., 2014). RSV, syncytial giant cells formed by non-ciliated bronchiolar epithelial cells with acidophilic cytoplasmic inclusion bodies, are the characteristic findings of the disease (Belknap et al., 1995).

Investigations have been carried out with serological tests such as Virus Neutralization Test (VNT), ELISA, Immunofluorescent Antibody (IFA) and Hemagglutination Inhibition (HI) for the prevalence of ruminant RSV and PI-3 agents (Franco et al., 2020; Tiwari et al., 2016). Immunohistochemical (IHC), Immunofluorescence (IF), Direct Fluorescent Antibody Technique, and Electron Microscope methods are currently used in the lung tissue of ruminant animals to show detailed RSV and PI-3 viruses (Aniță et al., 2015; Jarikre and Emikpe, 2017).

In this study, it was aimed to investigate the localization and distribution of RSV and PI-3 antigen in formalin-fixed, paraffin-embedded lung tissue sections in sheep and goat lung viral pneumonia cases by histopathological and immunohistochemical methods.

MATERIAL and METHOD

The study material consisted of 54 sheep and goat lung paraffin blocks that brought to Samsun Veterinary Control Institute from Samsun and surrounding provinces between 2016 and 2019, showing signs of viral pneumonia.

Histopatological method

Sections 5 µm in thickness were cut for histological examination. The slides were examined and photographed using. Sections 5 µm in thickness were cut and taken normal and poly-L-lysine slides. Normal slides stained with routine Hematoxylin-eosin for the histopathological examination. All slides were examined and photographed using light microscope (Leica DM 400B).

Immunohistochemical Method

Immunohistochemical staining was performed according to the Mouse and Rabbit Specific HRP / DAB IHC Detection Kit-Micro polymer (ab236466) kit procedure. Anti-Mouse RSV monoclonal (Cat NO: ab43812, Abcam, Boston USA) and anti-Goat polyclonal PI-3 (Cat NO: ab 28584, Boston, USA) were used. Sections taken from paraffin blocks to poly-L-lysine slides were washed in distilled water after deparaffinization and dehydration. Firstly, 3% H_2O_2 peroxidase block solution was dropped for 10 min. Proteinase K (ab64220) was

dropped for antigen retrieval and it was waited for 5 min. Then, the protein block solution was poured and kept at room temperature for 10 min. The sections were dropped 1: 100 anti-RSV and 1: 200 anti-PI-3 and left at room temperature for 1 hour. Subsequently, the Mouse Identification Reagent (Complementary) solution was added to the slides and incubated for 30 min and Goat anti-rabbit HRP-conjugate was dropped for 15 min. Slides stained by DAB (3,3'- diaminobenzidine tetrahydrochloride) for 1 min. After counter-staining with Mayer's hematoxylin, slides were closed by coverslips and evaluated under a light microscope (Leica DM 400B). The negative control slides were also stained according to the same procedure and PBS was used instead of the primer antibody. In the immunohistochemical scoring of sections, bronchial and bronchiolar epithelial cells and cells debris, peribronchial glands, interalveolar septum inflammatory cells, alveolar macrophages, and neutrophils were examined semi-quantitatively. Immunohistochemical staining scores were classified as mild (+), moderate (++) and severe (+++) expression according to the criteria determined by Yavuz and Dinçel (2020)

Statistical Analysis

Statistical significance of immunohistochemical scores was evaluated using IBM SPSS Statistics 25.0 software and Mann-Whitney's U test. The criterion for statistical significance was $p<0.05$. All values are presented as mean±standard deviation (median).

RESULTS

Histopatological Results

In this study, degeneration and desquamation of bronchial and bronchiolar epithelium, fibromuscular hypertrophy (Fig 1A), epithelialization, interalveolar septum cell infiltration (Fig 1C) were the most prominent microscopic changes in RSV and PI-3 infections. Lymphoid hyperplasia of the tissue around bronchi and bronchioles was detected in 17 %-25 % of RSV and PI-3 cases (Fig 1B).

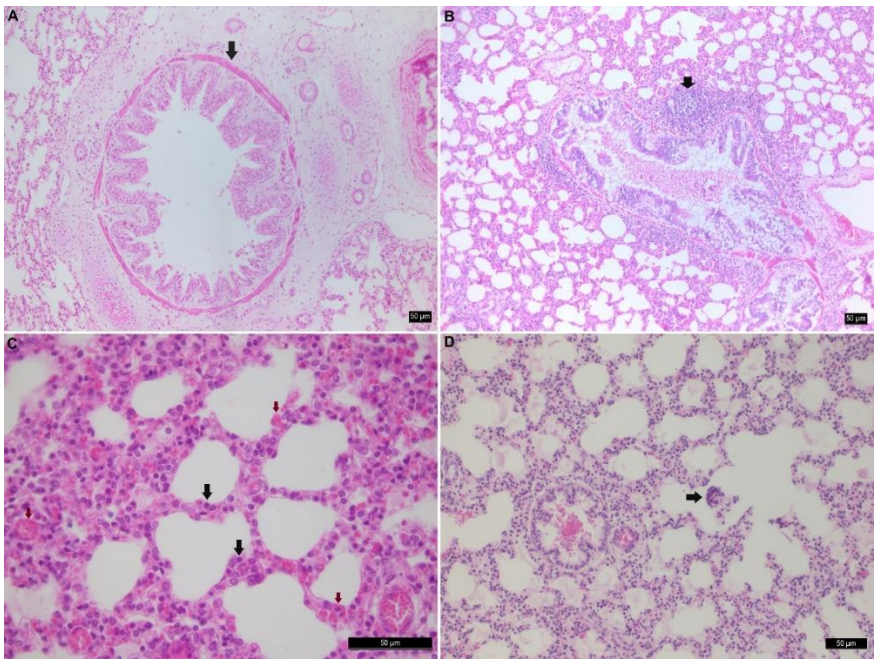

Figure 1. Lung sections, Hematoxylin-Eosin Staining. **A.** Fibromuscular hypertrophy around the bronchial (arrow). Bar: 50 µm **B.** Desquamation of bronchial epithelium and hyperplasia of lymphoid tissue around the bronchial (arrow). Bar: 50 µm. **C.** Inflammatory cells infiltration (black arrows) and hyperemia (red arrow) in the interalveolar septum. Bar: 50 µm. **D.**Syncytial cell (arrow) and inflammatory cells infiltration in the interalveolar septum. Bar:50 µm**.**

Bronchiolitis obliterans, which is characterized by the formation of organized fibrous tissue covered with a single layer of cuboidal epithelium in the bronchiolar lumen, could not be detected in any sheep and goat lung sections. Alveolar epithelization, characterized by the transformation of a monolayer cubic epithelium, was common. In many cases, hyperemia in the interalveolar septum, edema and alveolar macrophages were seen intensely in alveolar lumens. In seven sheep and goats of RSV positive cases, and four sheep and eight goats of PI-3 positive cases, syncytial cells were present in the lumen of the alveoli (Fig 1D). Neutrophilic infiltration in lung tissue was seen in a small number of RSV and PI-3 positive cases.

Immunohistochemical Results

In immunohistochemical staining, RSV antigen was detected in 50% of sheep and 54% of goats, and PI-3 antigen was detected in 40% of sheep and 50% of goats. Statistical evaluation of IHC scoring of sheep and goat RSV and PI-3 antigen in the bronchial and bronchiolar epithelium and cells debris, peribronchial glands, interalveolar septum inflammatory cells, alveolar macrophages are given in Table 1 and 2.

Table 1. Range of the histopathological findings in sheep and goat lung RSV and PI-3 antigen positive cases.

	RSV (+)		PI-3(+)	
Lesions	**Sheep**	**Goat**	**Sheep**	**Goat**
Degeneration and desquamation of the bronchial and bronchiolar epithelium	87%	69%	83%	75%
Fibromuscular hypertrophy	100%	100%	100%	100%
Lymphoid hyperplasia	20%	23%	17 %	25%
Thickening of the interalveolar septum	100%	100%	100%	100%
Epithelialization	80%	80%	83%	83%
Hyalin membrane	14%	8%	8%	17%
Bronchiolitis obliterans	0%	0%	0%	0%
Syncytial giant cell	47%	54%	33%	67%
Intraalveolar edema	40%	31%	17%	42%
Intracytoplasmic inclusion body	7%	8%	25%	8%
Alveolar macrophage	93%	92%	100%	100%
Neutrophil Infiltration	20%	31%	33%	33%
Hyperemia	87%	100%	100%	100%

Table 2. Statistical results of RSV and PI-3 antigen in sheep and goat lung tissue by IHC method.

	RSV(+) Sheep	RSV(+) Goat		PI-3(+) Sheep	PI-3(+) Goat	
Bronchial epithelial cells and cells in the lumen	2.25±0.21 (2.50)	1.53±0.35 (1.00)	0.114	1.75±0.30(1.50)	1.66±0.35(1.00)	0.843
Bronchial glands	1.93±0.30 (2.50)	1.46±0.36(1.00)	0.398	1.41±0.22(1.00)	1.33±0.15(1.00)	0.713
Bronchiole epithelial cells and cells in the lumen	1.62±0.25 (1.50)	1.69±0.32 (1.00)	0.914	1.58±0.37 (1.00)	1.58±0.15 (1.00)	0.932
In inflammatory cells in the interalveolar septal tissue.	1.43±0.32 (1.50)	1.84±0.33(2.00)	0.374	0.91±0.28 (1.00)	1.41±0.16 (1.00)	0.319
Alveolar macrophages	1.37±0.32(1.00)	2.15±0.29 (3.00)	0.121	0.75±0.25 (1.00)*	1.58±0.25 (1.00)*	0.028*

*Data presented mean value ± standard deviation (median), significant differences (P<0.05) marked with different superscripts.

RSV and PI-3 antigen in lung tissue of sheep and goats, bronchial and bronchiolar epithelial cells and cell debris, in peribronchial glands and cell infiltration interalveolar septum were not statistically ($p>0.05$) significant (Fig. 2 B-C-D). In alveolar macrophages, PI-3 antigen in goats was statistically stained more intensely in IHC staining than in sheep ($p<0.05$). RSV

antigen was found to be positive in the cytoplasm of neutrophils (Fig. 2A) in one sheep and two goats. RSV antigen was not identified in the syncytial cell cytoplasm. Immunopositive staining was detected in bronchial cartilage tissue in PI-3 positive cases.

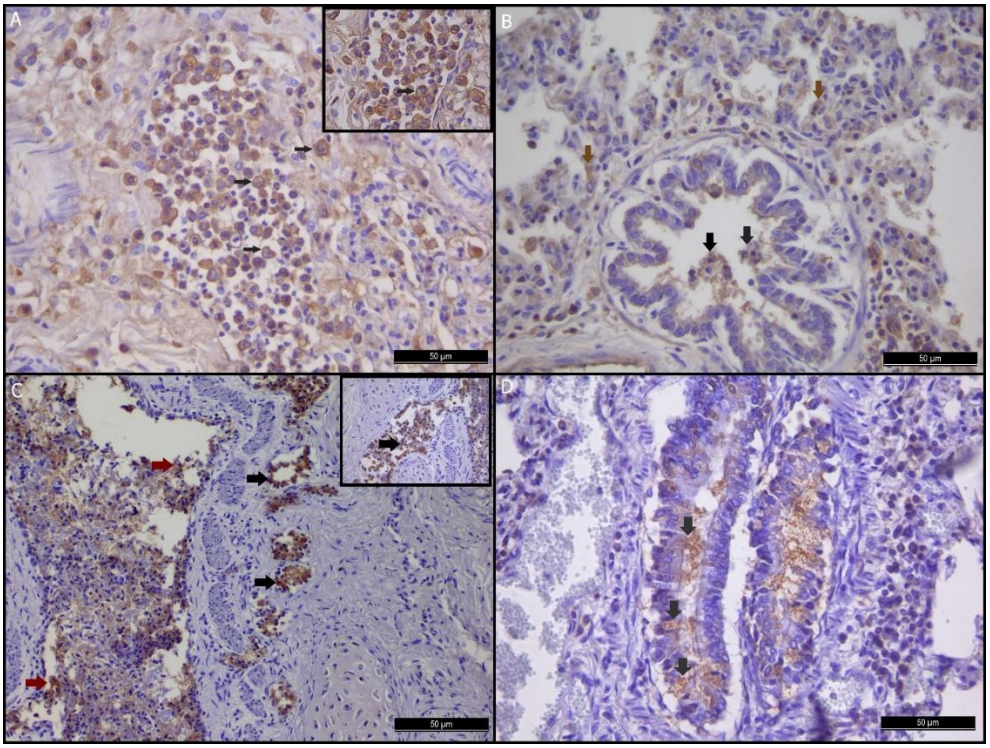

Figure 2. Immunohistochemical staining of sheep and goat lung's. **A.** Anti-RSV positive reactions in alveolar macrophages and neutrophils (arrows). Bar:50 µm. **B.** Anti-RSV positive reactions in the bronchiolar epithelium and cells debris (black arrows) and alveolar macrophages (red arrows). Bar: 50 µm. **C.** Anti-PI-3 positive reactions in the bronchial epithelium and cells debris (red arrows) and peribronchial glands (black arrows). Bar:50 µm. **D.** Anti- PI-3 positive reactions in bronchiolar epithelium and lumen (black arrows). Bar: 50 µm.

DISCUSSION

The anatomical structure of the lung, its mucociliary defense mechanism, lymphoid tissue associated with the mucosa, and phagocytic cells play an important role in defense against microorganisms (Nicod, 2005). Viral agents may suppress the mucociliary system in the airway epithelium and increase susceptibility to bacterial infection (Belknap et al., 1995; Bryson et al., 1991). In the study, lung paraffin blocks infected with natural RSV and PI-3 viruses, in which only viral pneumonia findings were detected, which are not complicated by bacterial agents, were preferred and the distribution of these viruses in the lung tissue was investigated by the IHC method.

BRSV occurs cytopathological changes in both ciliary and non-ciliary bronchiolar epithelial cells and types II pneumocytes (Masot et al., 1995; Viuff et al., 1996). Replication of the PI-3 virus is observed in epithelial cells of the respiratory tract and alveolar macrophages (Bryson et al., 1983). The agents cause viral pneumonia by both damaging the respiratory tract epithelium and inducing cytokines and chemokines in the lung tissue. RSV and PI-3 have histopathological findings such as bronchitis, bronchiolitis, alveolitis, degeneration, desquamation, necrosis or hyperplasia of the bronchiolar epithelium, thickening of the alveolar septum, lymphocyte and neutrophil infiltration, and syncytial cell in the lung tissue (Afshar and Terlecki, 1979; Caswell and Williams, 2007; Sacco et al.,

2014). In addition, it is reported that an increasing number of alveolar macrophages and exudates occur in RSV infections (Ceribasi et al., 2014; Sacco et al., 2014). In this study, histopathological findings caused by RSV and PI-3 factors were determined in sheep and goat lung tissue. As the infection progresses, an attempt to repair necrotic airways results in epithelial hyperplasia and bronchiolitis obliterans, also known as bronchiolitis obliterans (Caswell and Williams, 2007). In the study, bronchiolitis obliterans was not found in paraffin sections of sheep and goat lungs, since acute interstitial pneumonia findings were prominent.

In previous studies, it was determined that the areas where BRSV was detected in the lung by the immunohistochemical method were virus replication areas (Masot et al., 2000). In studies conducted with natural and experimental RSV infection of ruminants (Ceribasi et al., 2014; Jarikre and Emikpe, 2017; Masot et al., 2000; Viuff et al., 1996; Yener et al., 2005), bronchiolar epithelial cells were determined by the IHC method in type II pneumocytes, epithelial cells of bronchial glands, syncytial cells, alveolar macrophages, and exudate in the bronchial, bronchiolar, and alveoli lumen. In the study, it was determined that the intensity of RSV antigen staining in sheep and goats was statistically similar in bronchial and bronchiolar epithelial cells and cell debris, peribronchial glands and interalveolar inflammatory cells infiltrations. Also, unlike other studies, RSV antigen was determined to be positive in the neutrophil cytoplasm of one sheep and two goats. In this study, RSV antigen was not found in the syncytial cell cytoplasm in both sheep and goats, while the presence of RSV antigen in the syncytial cell cytoplasm of the IHC method was reported in the lung tissue of natural and experimental RSV infection of sheep and goats (Jarikre and Emikpe, 2017; Redondo et al., 2003).

In ruminant naturally infected with PI-3 virus, bronchial, bronchiolar, and alveolar epithelium, exudate in the lumen and syncytial cells were also identified by the IHC method (Çeribasi et al., 2012; Yener et al., 2005). In this study, similar to previous studies, PI-3 antigen was stained on bronchial and bronchiolar epithelial cells, peribronchial glands, interalveolar inflammatory cells in sheep and goats. In addition, PI-3 antigen was statistically significant in sheep and goat alveolar macrophages and stained more intensely in goats. Ceribasi et al. (2014) and Yener et al. (2005) detected PI-3 positivity in bronch cartilage tissue in cattle and goats, and in the study, PI-3 antigen was found in bronch cartilage in both sheep and goats.

PCR, Culture, Virus Isolation, Electron Microscopy, DFAT, IFAT, and IP techniques are used to determine the prevalence of RSV and PI-3 virus in naturally infected flocks in our country and in the World (Emikpe et al., 2019; Jarikre and Emikpe, 2017; Sharma et al., 2017; Tiwari et al., 2016). In previous studies, the immunohistochemical method (IHC) was used to determine the presence of RSV and PI-3 antigen in respiratory tract infections of ruminants and their distribution in the lung (Ceribasi et al., 2013; Haines et al., 1992; Yener et al., 2005). The immunohistochemical method is seen as an advantageous technique in reaching the diagnosis with the histological results of retrospective studies, although more time is needed to process formalin-fixed tissues. For the IHC method to be successful, tissue fixation and the preferred antiserum technique and quality must be good (Haines et al., 1992). In the study, it was determined that the prevalence of natural PI-3 infection with immunoperoxidase (IP) method in goats was lower than the study of Yener et al. (2005) and higher than the study of Çeribasi et al. (2012). In addition, the prevalence of RSV viral antigen was higher than the rate determined by the IHC method Ceribasi et al. (2013). The prevalence

of RSV in sheep herds in our country by the immunohistochemical method and its distribution and localization in lung tissue was presented for the first time with this study. The immunohistochemical method is thought to be an effective method that can be used to identify RSV and PI-3 virus from archive materials.

CONCLUSION

It was concluded that the localization of RSV and PI-3 antigens in sheep and goat lung tissue was detected similar by this study. In addition, it was determined that RSV and PI-3 antigens were common in sheep and goats and it was thought that vaccination should be done for protection.

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Ethical approval: This study was approved by the Animal Experiments Local Ethics Committee of Samsun Veterinary Control Institute Directorate (Approval no: 2019/3-09.05.19)

Conflict of interest: There is no conflict of interest between the authors

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Prevalence of pelvic fractures in cat and dogs: A retrospective study in 183 cases (2016-2020)

ABSTRACT

This study aims to classify radiographically diagnosed pelvic fractures in cats (n = 103) and dogs (n = 80). The pelvic bone resembles a box structurally, and as a result of the trauma of this bone, multiple fractures usually occur. Radiographs of cats and dogs exposed to various traumas were evaluated and classified as ilium, ischium, pubis, acetabulum, sacroiliac luxations and symphysis pubis fractures. The mean age of the cases included in the study was 16.9 months in cats and 19.2 months in dogs. Pelvic fractures were more common in females than males (♀=57.9%, ♂= 42.1%). In this context, it was constituted 18.7% ilium fractures, 12.6% pubis fractures, 18.9% ischial fractures, 11.3% acetabulum fractures, 29.2% sacroiliac luxation and 9% symphysis pelvis fractures of pelvic fractures. As a result, it was revealed that multiple fractures could be seen in traumas taken to the pelvic area and their rates.

Keywords: Cat, Dog, Pelvic fractures, Prevalence, Trauma

INTRODUCTION

Pelvis consists of the ilium, ischia, pubis, sacrum and first coccygeal vertebra. The junction of each pelvic bone is called the symphysis pelvis. Pelvic fractures are usually caused by falling from a height, traffic accidents, kicking, gunshot and bite injuries, and tumoral causes (Altunatmaz et al., 2004; Mesquita et al. 2009, Witte and Scott 2012; Bourbos et al. 2020). Pelvic fractures are common injuries in cats and dogs and constitute 20-30% of all fractures caused by trauma (Altunatmaz et al., 2004; Draffan et al. 2009; Mesquita et al. 2009; Stieger-Vanegas et al. 2015; Sadan et al. 2016). Pelvic fractures are generally classified as sacroiliac luxations, iliac wing, iliac body, acetabular, ischial and pelvic floor fractures. In pelvic fractures, the ilium is most affected (18-46%) (Altunatmaz et al., 2004; Harasen 2007; Stieger-Vanegas et al., 2015). In untreated cases, the degenerative joint disease develops because joint compliance will be impaired (Mesquita et al. 2009). On the other hand, dogs have difficulty in carrying body weight due to coxofemoral luxations and sacroiliac separations, which is considered a common cause of morbidity (Draffan et al. 2009). Concomitant injuries to other body systems, including life-threatening injuries, are also common and should be detected and treated in a timely manner. Soft tissue and organ damage are common in multiple pelvic fractures. Lower urinary and gastrointestinal system organs and peripheral nerves are mostly affected due to pelvic fractures (Ünsaldı 1995; Sadan et al. 2016).

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Research Article

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Radiographic examination of the pelvic is a standard diagnostic test to evaluate cases with suspected trauma. Shots are typically done in laterolateral, ventrodorsal, and oblique positions (Altunatmaz et al., 2004; Mesquita et al., 2009; Sadan et al. 2016). The pelvic is similar to a box in terms of its structure, so it is very likely that more than one bone will be affected during trauma; that is, only one bone may be affected or more than one pelvic bone may be affected (Denny 1978; Altunatmaz et al., 2004; Mesquita et al. 2009). However, the displacement of the fracture parts is generally not formed without 2 or 3 fractures due to the pelvic structure (Bourbos et al. 2020). The most important complications in pelvic fractures are damage to the pelvic structures and nerves, especially the sciatic nerve (Ünsaldı 1995; Meeson and Geddes 2017). When the damage takes shape, it should be treated conservatively or surgically (Houlton and Dyce 1994; Ünsaldı 1995; Mesquita et al. 2009).

This study aims to retrospectively of pelvic fractures in cats and dogs admitted to our clinic with pelvic trauma.

MATERIAL and METHOD

The material of the study consisted of a total of 183 cases, including 103 cats and 80 dogs of different ages, breeds and sex, brought to the Hatay Mustafa Kemal University Veterinary Health, Practice and Research Hospital between 2016-2020. In a total of 183 cases included in the study, 475 fractures were evaluated. In the first stage, radiographs of the cases were taken in ventrodorsal and laterolateral positions. After radiographic examining, pelvic fractures; ilium, ischia, pubis, acetabulum, sacroiliac separations and as symphysis pelvic fractures were categorized. All fractures except symphysis pelvic fractures were classified as right and left sides. Also, unilateral and bilateral incidence rates for bilateral pelvic bones were also presented. Fractures were named as traffic accidents, falling from a height, dog attack and the cause of unknown trauma.

RESULTS

A total of 183 cases, including 103 cats and 80 dogs, were included in the study. Seventy-seven of the animals affected by the trauma were male, and 106 of them were female. 39 (37.8%) of the cats were male, 64 (62.2%) of them were female, while 38 (47.5%) of the dogs were male, and 42 (52.5%) of them were recorded as female. The average age of cats was 16.9 months, and dogs were 19.2 months. Of the pelvic fractures were evaluated as 135 (73.7%) from traffic accidents, 34 (18.5%) from falling from a height, 1 (0.5%) from a dog attack, and 13 (7.1%) of unknown.

Table 1. Information on 183 cases that are taken into evaluation

Species	*Cases*	*The average age*	*Sex* M/F*	*Etiology** TA/FH/DA/U*	*İlium fracture left/right*	*İschii fracture left/right*	*Pubic fracture left/right*	*Acetabular fracture left/right*	*Sakroiliac separation left/right*	*Symphysis pelvis fracture*	*Fractures*
Cat	103	16.9	39/64	60/31/1/11	17/26	21/26	16/18	9/15	51/42	28	269
Dog	80	19.2	38/42	75/3/0/2	18/28	18/25	12/14	14/16	23/23	15	206
Total	183	18.05	77/106	135/34/1/13	35/52	39/51	28/32	23/31	74/65	43	475

*: M: Male, F: Female

**: TA: Traffic accident, FH: Falling from high, DA: Dog attack, U: Unknown

60 of the pelvic fractures in cats were caused by a traffic accident, 31 from falling from a height, 1 from a dog bite, and 11 from an unknown reason. In dogs, 75 of the cases were caused by a traffic accident, 3 as a result of a fall from a height, and 2 as a result of an unknown trauma. In 183 cases included in the study, 475 fractures were detected, 269 in cats and 206 in dogs. These; 35 left ilium fractures, 54 right ilium fractures; 39 left ischial fractures, 51 right ischial fractures; 28 left pubis fractures, 32 right pubis fractures; 23 fractures of the left acetabulum, 31 fractures of the right acetabulum; 74 were left sacroiliac separations, 65 were right sacroiliac separations, and 43 were symphysis pelvis fractures (Table 1). Of cats; left ilium fracture in 17, right ilium fracture in 26 (Figure 1a); 21 left ischial fracture, 26 right ischial fracture; 16 left pubis fracture, 18 right pubis fracture; 9 had left acetabulum fracture, 15 had right acetabulum fracture (Figure 1a);

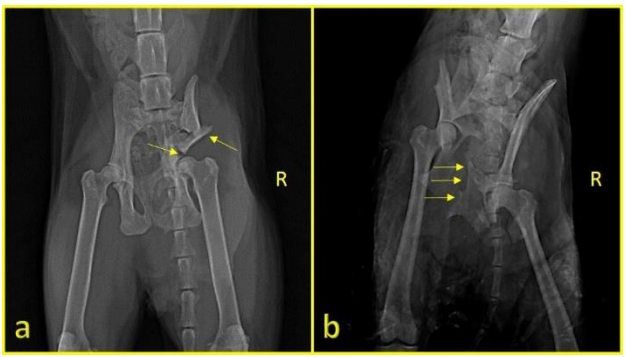

Figure 1a. Radiography of a cat with a fracture of the right ilium and right acetabulum in the pelvic **b)** Radiography of a cat with a symphysis pelvic separation in the pelvic

51 left sacroiliac separations, 42 had right sacroiliac separations, and 28 had symphysis pelvis fracture (Figure 1b); If the dogs; left ilium fracture in 18, right ilium fracture in 28; 18 left ischial fracture, 25 right ischial fracture; 12 had left pubis fracture, 14 had a right pubis fracture; 14 had left acetabulum fracture, 16 had right acetabulum fracture; 23 had left sacroiliac separations, 23 had right sacroiliac separations, and 15 had symphysis pelvic fractures.

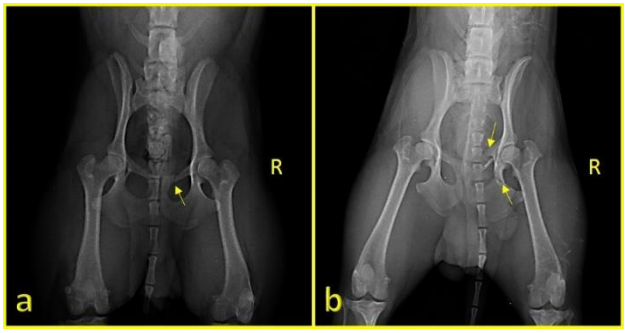

Figure 2a. Radiography of a cat with a single pelvic fracture (left ischial bone) **b)** Radiography of a cat with multiple pelvic fractures (right ischial bone and bilateral sacroiliac separation)

More than one pelvic bone was fractured in 136 of the 183 cases in cats and dogs. Of the 103 cases in total, 25 (24.2%) of the cats had a single pelvic bone (Figure 2a), and 78 (75.8%) had more than one pelvic bone (Figure 2b). In dogs, 22 of 80 cases (27.5%) had a single pelvic bone (Figure 3a), and 58 (72.5%) had multiple pelvic fractures (Figure 3b).

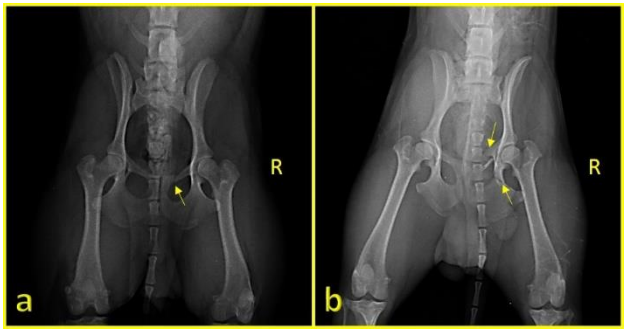

Figure 3a. Radiography of a dog with a single pelvic fracture (right os pubis) **b)** Radiography of a dog with multiple pelvic fractures (right ischial bone and right os pubis)

Bilateral fracture of the ilium in 1 (0.9%) of the cats, bilateral ischial fracture in 6 (5.8%) (Figure 4a),

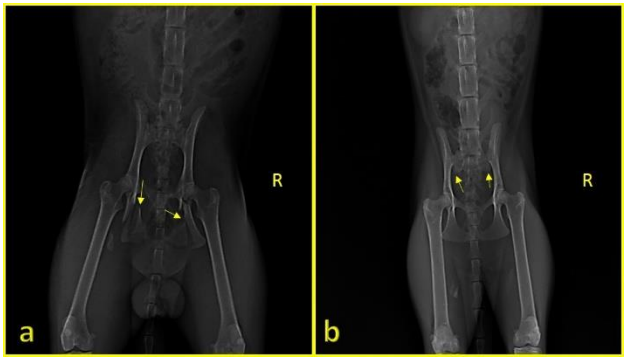

Figure 4a. Radiography of a cat with bilateral ischial fracture of the pelvic **b)** Radiography of a cat with bilateral sacroiliac separation in the pelvic

bilateral pubic fracture in 8 (7.7%), bilateral acetabular fracture in 1 (0.9%) and bilateral sacroiliac separations in 29 (28.1%) (Figure 4b); In dogs, bilateral ilium fracture in 7 (8.7%), bilateral ischial fracture in 5 (6.2%), bilateral pubic fracture in 5 (6.2%), bilateral acetabular fracture in 3 (3.7%) and 12 bilateral sacroiliac separations (15%) were detected (Table 2).

Table 2. Unilateral and bilateral fractures in cats and dogs

Species	*İlium fracture U/B****	*İschii fracture U/B*	*Pubic fracture U/B*	*Acetabular fracture U/B*	*Sakroiliac separation U/B*
Cat	42/1	35/6	18/8	22/1	35/29
Dog	31/7	33/5	16/5	24/3	22/12

***: U; Unilateral, B; Bilateral

DISCUSSION

Pelvic fractures, which are common in small animals, constitute 16% of all fractures in dogs and 25% in cats (Bourbos et al., 2020; Cinti et al., 2020). Mesquita et al., (2009) reported that traffic accidents were the most common cause of pelvic fractures. Meeson and Geddes (2017) unveiled that motor vehicle accidents are the most common cause of trauma, and they argued that the geographical region inhabited has also an effect on this condition. The same researchers stated that this rate might be high in settlements close to the metropolis, especially in stray dogs. In parallel with these determinations, the reason for 73.7% of the cases evaluated in our current study is traffic accidents. Most of the fractures in cats and dogs are caused by traffic accidents. Falling from a height is the second reason for fractures in cats.

Sadan et al., (2016) stated that most pelvic fractures are seen in healthy animals under the age of three. Moreover, Bourbos et al., (2020) also revealed that pelvic fractures are mostly seen in young animals and associated with the walking habits of the animals. On the other hand, Bennet (1975) and Ünsaldı (1995) reported that the animals with pelvic fractures were between the ages of 1-3. In our study, the average age of pelvic fractures was observed as 16.9 months in cats and 19.2 months in dogs, consistent with the studies mentioned above.

Johnson and Hulse (2005) emphasized that there is no race, age or gender predisposition in pelvic fractures which occur in small animals.

Bennet (1975) stated that pelvic fractures are detected more in male cats compared to female cats. On the contrary, Ünsaldı (1995) suggested that pelvic fractures are more common in females. In our study, a diagnosis of pelvic fracture was made in many races rather than specific races. In other words, no racial predisposition has been determined. It has been found that the ages of affected animals are close to each other in cats and dogs. In terms of gender, it was noted that fractures were more common in females than males in both dogs and cats (Table 1).

Ünsaldı (1995) reported that 80% of cases with pelvic fractures had multiple fractures. Similarly, the incidence of such fractures is 75.8% in cats and 72.5% in dogs, consistent with Unsaldı's results (1995).

Bookbinder and Flanders (1992) stated that the most common pelvic fracture in cats is pelvic floor fractures, and these fractures accounted for 90% of cases. Sadan et al., (2016) unveiled in their study that 54.5% of cats and 59.6% of dogs had unilateral pubic fractures. In our study, unilateral pubic fractures were detected in 17.4% of cats and 20% of dogs.

DeCamp (2005) stated that the most common fracture is the ilium fracture. Furthermore, Bouabdallah et al., (2020) reported in their study that the rate of ilium fractures among pelvic fractures was 35.7%. In the present study, we found that the rate of ilium fractures among

pelvic fractures was 20.8%. Bouabdallah et al., (2020) reported that the rate of sacroiliac separation among pelvic fractures was 59.5%.

Johnson and Hulse (2005) suggested that sacroiliac separations should be surgically treated. In contrast, there are also studies suggesting conservative treatment for sacroiliac separations (Mesquita et al., 2009). On the other hand, DeCamp (2016) emphasized that there could be neurological deficits in sacroiliac separations. In thecurrent study, sacroiliac separation was mostly diagnosed in cats (34.5%). In dogs, this rate remained at 22.3%. It has been reported that sacroiliac separations are mostly unilateral (Aksoy et al., 2005). In the present study, 29 of sacroiliac separations were bilateral (28.1%) in cats, 35 unilateral (33.9%), 12 bilateral (15%) in dogs, 22 unilateral (27.5%). These results are compatible with the literature mentioned above.

The incidence of acetabular fractures varies between 14% and 43% (Hardie et al., 1999; Boswell et al., 2001; Mesquita et al. 2009). Bouabdallah et al. (2020) reported the incidence of acetabulum and pubis fractures to be 21.4% in their study. In our study, these rates are 11.3% and 12.6%, respectively. The rate of formation of ischial fractures is 18.9% (Bouabdallah et al., 2020). Sadan et al., (2016) revealed that the incidence of unilateral ischial fractures is 67.9% in dogs and 64.3% in cats. In our study, unilateral ischial fractures between the pelvic bones were observed as 13.1% in cats and 16.0% in dogs.

Bourbos et al., (2020) unveiled that symphysis pelvic fractures are rarely occured. Sadan et al., (2016) emphasized in their study that symphysis pelvic separations are more common in cats than in dogs (15.3% in cats; 5.4% in dogs). Among the cases included in our study, symphysis pelvic fracture occurred in 27.1% in cats and 18.7% in dogs. The incidence of pelvic fractures is only 9%.

Most veterinary orthopedic surgeons recommend that most pelvic fractures in cats and dogs need surgery. However, surgical interventions may be disrupted due to financial constraints, chronic fractures, or veterinarians not having enough experience in orthopedics (Bouabdallah et al., 2020). In addition to these, radiographic evaluations in pelvic fractures are of great importance in planning the treatments to be applied.

CONCLUSION

In cats and dogs, multiple fractures are encountered in the pelvic bone, especially as a result of motor vehicle accidents and falls from height. In addition to protecting the digestive and excretory system organs with its box-like structure, it should be kept in mind that this bone may be damaged due to its neighbourhood with the sciatic and femoral nerve, especially in sacroiliac joint separations. As a result, of pelvic fractures were observed to cause 18.7% ilium fractures, 18.9% ischial fractures, 12.6% pubis fractures, 11.3% acetabulum fractures, 29.2% sacroiliac separations, and 9% symphysis pelvic fractures.

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A review of endo- and ecto-parasites of equids in Iraq

Review Article

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ABSTRACT

This article summarizes the findings of the majority of Iraqi studies and lists the most common zoonotic and non-zoonotic parasites. As a result of the proper reporting of protozoa, helminthes, hard and soft ticks, a large number of parasites have been described and distributed throughout Iraq. *Toxoplasma*, *Theileria*, *Babesia*, *plasmodium*, *Anaplasma*, *Microfilaria*, *cryptosporidium*, *giardia*, *Eimeria*, *Balantidium*, and *Entamoeba* were among the protozoa that were frequently recorded. Helminths of the genera *Dicrocoelium*, *Habronema*, *Echinococcus*, *Dictyocaulus*, *Trichostrongylus*, *Parascaris*, *Oxyuris*, *Cyathostomum*, *Anoplocephala*, *Setaria*, and *Fasciola* have been reported to affect all types of horses, whether they are on grass or in stalls. Hard and soft ticks, as well as Sarcoptes, were the only ectoparasites that were frequently observed. Parasitic fly species from the *Gasterophilus* genus were also discovered. In Iraq, parasitic infections in horses are common and caused by a variety of parasites, posing a health risk and causing significant economic losses. Emaciation, fever, pale mucosal membranes, jaundice, colic, and diarrhoea are all symptoms of infected equines with piroplasms, which are also accompanied by anemia, leukocytosis, and hyperbilirubinemia. Parasitic infections are linked to a number of risk factors (age, gender, activity, location, and season), and zoonotic parasites pose a greater risk to horsemen. All parasitic infections should be treated intramuscularly, with the exception of ivermectin, which should be taken orally. Not only are coprological examinations to identify distributed species and chronic infections, but also modern methods are used to control vectors and conduct further research.

Keywords: Equine protozoa, piroplasma spp., Ticks, Equine helminths, Iraq

INTRODUCTION

Horses (*Equus ferus caballus*), donkeys (*Equus africanus asinus*), mules, zebras (*Equus zebra*), and other animals with similar characteristics are classified as "equine." The equine family serves a variety of functions in the agricultural economy, including transportation of farm products, firewood, water, recreational activities, and farm ploughing. Horses, donkeys, mules, and zebras are infected with a variety of parasite species that differ in life cycles, pathogenicity, epidemiology, and the drugs used to combat parasite infections (Scoles and Ueti 2015). Infection in these animals can be single or mixed, resulting in a variety of clinical signs. Intestinal, blood, and ectoparasite infections are all possible. Ectoparasites are a common target for many parasite species. Anemia, fever, diarrhea, colic, hemorrhage, pale mucosal membranes, jaundice, hyperbilirubinemia, emaciation, and death are some of the diseases that the infective parasites can cause in animals. Due to significant economic losses worldwide, the identified gastrointestinal (GI) and equine piroplasmosis (EP) parasites are the most serious problems for equids (Raue et al., 2017, Camino et al., 2019, Zhao et al., 2020).

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Babesiosis is a globally distributed infectious disease that causes equine babesiosis. It can be acute, sub-acute, or chronic. Equine piroplasmosis is a disease that is found in most subtropical and tropical regions of the world. The distribution and seasonal activity of biological vectors (ticks) that transmit and cause equine piroplasmosis (*Theileria* spp., *Babesia* spp., and *Anaplasma* spp.) have been linked (Scoles and Ueti 2015). Toxoplasmosis risk factors have been linked to equine protozoal myeloencephalitis (EPM), but clinical signs are uncommon. A neurologic disease caused by two apicomplexan protozoal parasites (*Neospora hughesi* and *Sarcocystis neurona*), which were previously unknown and limited to the autumn season (James et al., 2017). *Toxoplasma gondii* parasites were isolated from slaughtered horses destined for horsemeat as a possible source of human infection. One of the major risk factors for human toxoplasmosis is eating undercooked or insufficiently cured meat. In Serbia, *T. gondii* infection was found in slaughter horses, and viable *T. gondii* type III was discovered and isolated (Klun et al., 2017). Equine piroplasmosis is a tick-borne or transmitted disease of horses caused by single or mixed infections of two hemoprotozoal parasites (*Theileria equi* and *Babesia caballi*) (Hodgkinson, 2006, Ueti et al., 2008). Several infected equids are parasite carriers who show no clinical signs, making them one of the most dangerous factors in parasite transmission to other equids (Sunday Idoko et al., 2020). *T. equi* is thought to have a wider distribution and is more pathogenic than *B. caballi* in endemic (tropical and temperate) areas. *B. caballi*-infected horses have more severe hemolytic anemia; however, *T. equi* infections are mostly associated with leukocytosis (Camino et al., 2019). Equine piroplasmosis is a disease that affects horses, donkeys, mules, and zebras, as well as DNA parasites in camels and dogs. With *T. equi* infection, infected equids remain carriers for a long time, whereas infection with *B. caballi* is detected after only a few years (Onyiche et al., 2019). Horses and donkeys are infected with blood-parasites (*T. equi* and *B. caballi*) that can cause serious clinical diseases and have genetic diversity, as shown by phylogenetic analyses. In Jilin, China, two *T. equi* genotypes (A and E) were discovered (Zhao et al., 2020). Equid roundworms (Parascaris equorum) are uncommon but can affect young animals' growth and cause intestinal and respiratory symptoms. Other parasitic nematodes, such as *Oxyuris equi* (pinworm), are primarily irritants or nuisances, but persistent infection in some animals can lead to destruction around the perineum and tail head (Raue et al., 2017). There are three *Eimeria* species found in equids. *E. uninugulata* and *E. solipedum*, both named after horses, are considered invalid; only *E. leuckarti* is considered valid. Only oocytes and gamonts of *E. leuckarti* have been found recently; no asexual stages have been found. *E. leuckarti* infection has no clinical significance and is considered coincidental (Dubey and Bauer, 2018). Donkeys, unlike mature horses, are susceptible to infection by large strongyle (*Strongylus*) species and *Dictyocaulus arnifieldi*. Adult horses and donkeys can be significant sources of pasture contamination, and when they return from overwork, they can cause health problems and poor nutrition, as well as poor performance and physical condition, as well as serious pathological conditions such as severe diarrhea, colic, and even death (Brady and Nichols, 2009; Sazmand et al., 2020). The use of macrocyclic lactones (ML), ivermectin, and moxidectin reduced large strongyle infections in equine populations, while strongyle resistance to ivermectin, doramectin, and fenbendazole increased. In adults and small animals, the previous anthelmintics were highly effective against pathogenic larval stages (Hodgkinson, 2006). Cestocides, which were once widely regarded as pathogenic, could be to bear responsibility for the decrease in anoplocephalid infection (Raue et al., 2017, Hodgkinson, 2006).

Anoplocephala perfoliata is a common parasite that affects horses and donkeys. It has an indirect life cycle that involves an oribatid mite. While common trematodes (*Fasciola hepatica*) that affect donkeys, the adult fluke of *A. perfoliata* is found in the bile ducts and clinical signs are not usually detected in infected donkeys, the intensity was mostly in donkeys over the age of 8 years (Matthews and Burden, 2013). Coproscopical methods have been widely used in veterinary parasitological diagnostics, and their sensitivity (Se) and egg recovery rate have been validated (Becker et al., 2016). Different species of Ixodides were previously recorded as hard ticks with a cosmopolitan distribution, especially in moderate regions among the Ixodidae. Agents are transmitted by ticks belonging to the genera *Rhipicephalus* spp., *Hyalomma* spp., and *Dermacentor* spp. Because they transmit various protozoal, bacterial, and viral agents to humans and animals, they are of enormous medicinal and veterinary importance. Tick-borne protozoans may be transferred vertically across generations as well as transstadially and intrastadially within one tick generation. The protozoal pathogen was efficiently acquired by *Rhipicephalus microplus* male ticks during acute and chronic infections, and they transmitted it intrastadially to naïve horses and persistently infected horses, indicating that they should be targeted for disease prevention (Scoles and Ueti 2015, Ueti et al., 2008). Many articles about equid parasites have been published in Iraq, describing the various infections that are endemic and widespread across the country's governorates. Because this breed is primarily used for presentations, Iraqi Arabian horses have a high value among Arabian horses, which is primarily associated with their exterior conformation. The body shape of Iraqi Arabian horses is characterized by an increase in body length when compared to body weight, as well as a short back line. The Iraqi Arabian horse excels in sporting events and has a high lyse for long distances (Mohamed, 2017). The parasites infection, particularly gastrointestinal parasites, was encountered in 100% of the horses and donkeys, and the factors cold, hot, and rainy season had an effect on the distribution of infections (Albadrani and Aldelami, 2009; Esmaeel, 2010; Wannas, 2012; Zangana et al., 2013).Equine piroplasmosis (EP) is a tick-borne protozoal disease of horses, donkeys, mules, and zebra that has been infected by blood protozoal parasites (*Theileria equi* or *Babesia caballi*) in Iraqi horses (Fig. 1,2). It results in significant economic losses for the equids (Khalid Jabar et al., 2019; Sray et al., 2019). The EP is characterized by fever, jaundice, anemia, loss of appetite, and, in some cases, sudden death, and is found in most regions of Iraq (Alsaad, 2014). In equids, four 18S rRNA genotype clades were found for *T. equi* (A, B, C, and D) and two for *B. caballi* (A and B) (Aziz et al., 2019). Infected equids may be long-term carriers of these protozoal parasites (Alsaad et al., 2012). Equine anaplasmosis (*Anaplasma phagocytophilum*) is a bacterial/protozoan disease diagnosed in horse blood and transmitted by various ticks. Some reservoir hosts of agents, such as *Hyalomma* spp. and *Rhipicephalus* spp., also play a role in their transmission (Albadrani and Al-Iraqi, 2019). Other coccidian parasites have been infecting animals, such as *Toxoplasma gondii* in horses (Mikaeel and Al-Saeed, 2020; Hussain, 2011), Microfilaria and *Plasmodium* sp. in horses and Donkeys (Hadi and Atiyah, 2014), and only Microfilaria (*Setaria* spp.) in horses (Suleiman et al., 2020). Equine babesiosis is a tick-borne illness that affects horses and is caused by the protozoan parasites (*T. equi* and *B. caballi*) (Albadrani and Al-Iraqi, 2019). Fever, icterus, pale mucous membranes, and hemoglobinuria are among the symptoms of equine babesiosis, which may lead to a loss of condition and even death (Alsaad et al., 2010). The protozoan parasites are naturally transferred from host to host by hard tick vectors, and vertical transmission of *T. equi* and *B. caballi* infection in utero has been recorded (Khalid Jabar et al., 2019). *Babesia* may infect a fetus at any stage of

pregnancy, and it can also induce abortions in enzootic areas. Infected horses may carry *T. equi* infections for the rest of their lives, while infected equids can carry *B. caballi* for up to four years. Furthermore, unlike *B. caballi, T. equi* is not totally cleared from horses' blood following natural recovery or therapy (Alsaad et al., 2012; Aziz and AL-Barwary, 2020). In certain cases, the clinical indications of piroplasmosis infection are non-specific and varied. The sickness may be acute, chronic, or sub-clinical, with the diseased animal aiding in the transmission of the agents. However, in chronic and sub-clinical cases, it is sometimes difficult to identify the protozoans in blood smears of carrier equids. High fever, jaundice, petechiation, depression, anemia, dyspnea, sweating, eyelids, decreased appetite, colic, incoordination, and distal limb edema are all symptoms of an acute infection (Al-Rammahi et al., 2020; Al-Saad, 2009). Infections with zoonotic gastrointestinal protozoa (*Cryptosporidium* spp., *Giardia* spp., and *Eimeria* spp.) have been found in wild and farmed equines, suggesting that they might be sources of contamination. People may get infected by direct contact with horsemen and other humans, as well as recreational horses (Altaee et al., 2014). *Cryptosporidium* spp. and *Giardia doudenalis*, which cause watery diarrhea in horses and foals and must be prevented from spreading to people and causing substantial losses, were among the most zoonotic protozoan infections (Fig. 3 and 4). Horse *Cryptospridium* oocytes are 4-5 microns in diameter, have a tiny spherical form, and are pink in color (Mahdi and Ali, 2002; Butty, 2011; Moosa, 2019). *Eimeria leuckarti* is more frequent in foals, although the prevalence of other species is unknown (Kalef, 2015).

Microscopical assays, including Giemsa stain, direct smear, sedimentation, zinc sulphate flotation, Sheather's sugar saturated flotation method, acid ether technique, formalin-ether sedimentation concentration method, modified Ziehl-Neelsen method, cellophane tape methods, and the McMaster method, are the most commonly used diagnostic methods in Iraq. Although serological approaches have lower sensitivity than contemporary tests, they are beneficial in chronic infections. Molecular methodology is a highly important and reliable diagnostic procedure for the identification of hemoparasite species (Albadrani and Aldelami, 2009; Esmaeel, 2010; Wannas, 2012; Zangana et al., 2013; Khalid Jabar et al.,2019; Albadrani and Al-Iraqi, 2019; Mahdi and Ali, 2002; Abdul-Majeed and Al-Saad, 2006; Faraj et al., 2019; Alali et al, 2021b).

Mange mites, lice, and ticks are among the ectoparasitic diseases that pose a significant concern to humans, including horse owners, farmers, and even pigeon fanciers, and may transmit a variety of zoonotic parasites (Alali et al., 2020a; Faraj, 2013; Hasson, 2016). Ticks are distinguished by different species within the same genus and different distributions of disease vectors (ticks), resulting in the most prevalent diseases and widespread distribution in northern Iraq (Mustafa, 2019; Aziz and AL-barwary, 2019), central Iraq (Mohammad, 2015), and southern Iraq (Hajeel and Abd Alfatlawi, 2019).

Helminth infections in horses and donkeys might appear healthy, and donkeys seldom exhibit indications of significant illness (weight loss, diarrhea, poor condition, or colic), but horses do (Zangana et al., 2013). Many species of horse helminthes were found in fresh vegetables, including *Echinococcus* sp., *Oxyuris equi*, *Habronema* sp., *Parascaris equroum*, and *Strongyloides westrei*. This is due to urban gardeners increasingly irrigating their agriculture regions with waste water, and the spread of parasites in the north and middle of Iraq may be due to the reuse of equine feces for man (Hadi, 2011). The giant strongyle (*Strongylus vulgaris*) is the most pathogenic strongyle species in Iraq, causing thrombosis and thromboembolic colic. *Strongylus vulgaris* is more common and harmful among equid

parasites, and it is widely dispersed among grazing horses, posing a danger to equine health across the globe (Zangana et al., 2013).

Draught horses were infected with gastrointestinal and lung worms, which could be recognized by coprological tests. Many kinds of nematodes and cestodes were found. Emaciation, pale mucous membranes, rough coat, loss of appetite, black spots on the gum and lip, diarrhoea and/or constipation, colic, worms with mucoid feces, anal pruritis, moist raised, high heartbeat, and coughing are all symptoms of helminth infection. Worms infect diseased horses, causing macroscopical and histological lesions in one or more organs (Abdul-Majeed and Al-Saad, 2006; Esmaeel, 2010). *Gastrophilus* species, particularly *G. intestinalis*, are gastrointestinal parasites that adhere to the stomach's surface. The pathophysiology of the larvae is linked to inflammatory reactions (Daoud et al., 1989). This study have been conducted to summarize the previous studies in field of equids of Iraq.

MATERIAL and METHOD

For the survey evaluation, electronic databases of endo, ecto, and blood-borne parasites in equids in Iraq were obtained for the survey. Dissertations, Google Scholar, Research Gate, Academia, and the official site of higher education and scientific research (https://www.iasj.net) are all examples of references. The references were arranged in one table by type of infection (external, internal, tissue, and blood parasites) and by date of publication (oldest to newest) as well as type of host, methods of diagnosis, number of specimens, percentage of infection, province, genera and species of agents, and provinces. All relevant papers were found by searching the titles of publications using the phrases (equids, horse, donkey, ecto-, endoparasites, and hemoparasites). The relevant publications were evaluated, and important information about equids was chosen and provided, including author information and publication year (Table 1).

Table.1. A summary of the prevalence of parasitic infections in Iraqi equids.

Host type	ectoparasite/protozoa	helminth	Diagnostic Technique	Sample Size	Prevalence (%)	Province	Reference
Horses	*Boophilus annulatus*, *Hyalomma detritum*, *Hyalomma anatolicum anatolicum* *Rhipicephalus turanicus*, *Rhipicephalus sanguineus sanguineus*	-	Microscopic examination	----	6.6%, 20%, 40%, 26%, 6.6%	Nineveh	Al-Moula and Rahemo, 2004
Drought horses	*1- Tick species Rhipicephalus sanguineus , Boophilus annulatus and Hyalomma anatolicum excavatum* *2- Theileria equi, , Babesia caballi, Mixed infection* *3- Sarcoptes scabiei*	-	Microscopic examination	21	1- 27.7% 2- 15.5%,14.4% , and 10% 3- 16.6 %	Baghdad	Faraj, 2013
1-Horses 2-Donkeys	Ixodid ticks *(Hyalomma anatolicum Rhipicephalus* (*Boophilus*) *annulatus*)	-	Microscopic examination	1- 10 2- 5	50% 20%	Baghdad, Wasit, Babil, Al-Diwaniya, Al-Muthana, Al- Najaf Al-Ashraf, Kerbala, Missan, Basrah and Thi Qar.	Shubber et al., 2014
1-Horses 2-Donkeys	Ixodid ticks1-*(Hyalomma anatolicum, H. scupense and Rhipicephalus annulatus)*, *2-(Rhipicephalus annulatus)*	-	Morphological features	1- 8 2- 6	1-1(12.5%) 2-1(16.7%)	central region of Iraq	Mohammad, 2015
Equine (horse, mule	*Boophilus annulatus* *Boophilus microplus* *Hyalomma marginatum* *Hyalomma excavatum*	-	Microscopic examination	349 equids	39.8%, 16.3%, 12.2%, 10.2%, 21.4%	Erbil	Aziz and AL-barwary, 2019

donkey and pony)	*Rhipicephalus turnicus*						
Equids	*Hyalomma marginatum marginatum, H. anatolicum exacavatum), Boophilus microplus, B. annulatus Rhipicephalus turanicus)*	-	1-conventional 2- multiplex polymerase chain reaction technique (c-PCR, m-PCR) targeting 18S rRNA gene	349	1-98% 2-20%	Erbil	Aziz and AL-Barwary , 2020
Horse	Mange (*sarcoptes scabei var equi*)		Morphological characteristics	72	1.4%	Diyala	Hasson, 2016
Foals	*Babesia equi* *Babesia caballi*	-	c-ELISA test	105 Blood	81.11% , 18.88%	Mosul	Al-Saad, 2009
Drought horses	A- *Babesia equi* B- *Babesia caballi*	-	c-ELISA	115	A-78/90, (86.58%) B-49/90 (54.39%)	Basrah	Alsaad et al.,2010
1-Horses 2-Donkeys	A-*Babesia equi* B- *Babesia caballi*		c-ELISA	1-(46) horses 2-(45) donkeys	A-1-33(71.73%) horses and 19 (42.22%)donkey B- 9(19.56%) horses and 2 (4.44%)donkeys	Mosul	Alsaad et al., 2012
Newborn foals	*Babesia equi*		1-Microscopical 2-Competitive c-ELISA	62	1-3/62(53.22%) 2-33/33(100%)	Mosul	Alsaad, 2014
Horse, Mule Donkey, Pony	*Theileria equi , Babesia caballi* Mixed infection		1-Microscopic examination 2-c-ELISA	Total 349 blood	1- Total=10.6% 8.3%, 1.7%, 0.6 2-otal=38.97% 20.9%, 11.2%, 6.9%	Erbil	Khalid Jabar et al.,2019
Horse, Mule Donkey, Pony	*Theileria equi , Babesia caballi* Mixed infection		mPCR	136 blood	Total= 55.88% 41.91%, 8.82%, 5.15%	Erbil	Aziz et al., 2019
Dragging horses	*Theileria equi and Babesia caballi*		1-Microscopical 2-Molecular (18s rRNA gene)	150	1-25/150 (16.66%) 2-9/25(36%)	Baghdad	Faraj et al., 2019
Horse	*Theileria equi*		-c-ELISA -Polymerase chain reaction (PCR).	130	- 36.92% -5.38%	Baghdad, Al-Qadisiyah, Wasit	Sray et al.,2019
Horse	*Theileria equi* (equine theileriosis)		-Polymerase chain reaction (PCR).	110 blood	38.18%	Al-Najaf	Al-Rammahi et al., 2020
Horses	*Anaplasma phagocytophilum*		Blood smears or buffy coat smears	45 blood smears	33.3 %	Mosul	Albadrani and Al-Iraqi, 2019
Horses	*Toxoplasma gondii*		Indirect ELISA PCR	62 Blood	17.7% , 18.2%	Duhok	Mikaeel and Al-Saeed, 2020
Donkey	*Toxoplasma gondii*		Latex agglutination test, Modified latex agglutination test , 2-mercaptoethanol test and Indirect ELISA test (Indirect IgG ELISA)	52 Blood	46.15 %, 8.33%, 91.67 %, 22.72%	Mosul	Hussain, 2011
Horse Donkey	*Plasmodium* sp.	Microfilaria	Microscopic examination of blood smear	54 blood	11.11% 5.55%	Baghdad university	Hadi and Atiyah, 2014

Horses	*Cryptosporidium muris*		Direct smear method, Modified acid fast stain and then formalin-ether sedimentation	25	3(12%)	Basrah	Mahdi and Ali, 2002
Horse	*1-Cryptosporidium sp., 2-Giardia doudenalis*		Wet mount, Flotation, lugol's iodine, Modified Ziehl Nelsecn (hot), Giemsa	107 fecal	Total=19.63% 27.10, 19.63	Nineveh	Butty, 2011
Horses	1-*Giardia spp.*, 2-*Cryptosporidium spp.*		1-Direct wet smear, 1-Lugol's iodine smear 2-Modified Ziehle Nelseen stain.	180 fecal (92 horses,	1- 4.45% 2- 64.13%	Baghdad	Altaee et al., 2014
-Adult Drought Horses -foals	*Cryptospridium sp. oocyst*		Microscopical examination (Modefied acid fast stain and Lugol's iodine stain)	50 fecal	30% (4%, 26%)	Mosul	Moosa, 2019
1-Draught horses. 2-Fourosia club horses	*Eimeria* oocysts		Direct method a Flotation technique.	Total 369 fecal 136 , 233	Total= 20.32% 30.14%, 14.59%	Baghdad	Kalef, 2015
Horse	-	*Gastrophilas intestinalis*	By Grossly	1-horse	------	Mosul	Daoud et al.,1989
Draught stallions	-	*Large strongyles, Parascaris equorum, Small strongyles (Cyathostomins), Dictyocaulus arnfieldi, Strongyloides westeri, Trichostrongylus axei, Oxyuris equi, Anoplocephala perfoliata, Parapnocephala mamillana and Gasterophilus intestinalis*	Flotation,sedimentation and Baermann technique	150	125(83.33%)	Mosul	Abdul-Majeed and Al-Saad, 2006
Draught stallions	-	*Large strongyles, Parascaris equorum, Small strongyles (Cyathostomins), Strongyloides westeri, Trichostrongylus axei, and Oxyuris equi*	Comparison treatment Between ivermectin(Oral paste 0.2mg/kg) and Oxybendazole(Oral suspension 10mg/kg)	20	Ivermectin in 14 days, while Oxybendazole 21 days	Mosul	Al-Saad and Abdul-Majeed, 2006
Drought horses	-	*Strongylus spp, Oxyuris equi* and *Parascaris equorum*	-Flotation method, Sedimentation method. -Single dose of mixture of ivermectin and Closantel	19 fecal	31.58%, 15.75%, 10.52%	Mosul	Albadrani and Aldelami, 2009
Native Donkeys	*Habronema musca,*	Large strongyles *Strongylus* spp., *Triodontophorus* s	Coprological examinations	70 fecal	70%, 36.6%, 33.3%, 33.3%, 20%, 13.3%,	Mosul	Esmaeel, 2010

		pp., Small strongyles (caythostomines), *Trichostrongylus axei, Parascaris equorum, Dictyocaulus arnfieldi Strongyloides westeri, Oxyuris equi, Gastrodiscus* spp *+Dicroceolium* sp p.			10%, 10%, 6.6%, 3.3%.		
Horse		*Oxyuris equi, Habronema sp. Parascaris equroum, Strongyloides westrei*	Sedimentation	60	27(45%),27(45 %)19(31.6%), 18(30%)		Hadi, 2011
1- Horses 2-Donkeys	*Cryptosporidium* spp., *Balantidium coli* and *Eimeria* spp. and *Entamoeba coli*	*1-Strongylidae, Parascaris equorum, Strongyloides westri, Trichostrongylus axei, Oxyuris equi, Strongylidae, Parascaris equorum, Strongyloides westri, Trichostrongylus axei, Oxyuris equi, Dictyocaulus arnfieldi,*	Flotation method, Sedimentation method Direct smear method.	100: fecal 1- 44 2- 56 100% In 1-& 2-	1- 50%, 40.90%, 22.72%, 25%, 11.36%, 0.45%, 15.90%, 6.81% 2- 57.14%, 2.14%, 28.57%, 7.85%, 17.85%, 7.85%, 19.64, 17.85%, 10.71% , 3.57%	Al Diwaniyah	Wannas, 2012
Horses	*Eimeria leukarti*	*Strongylus vulgaris, Parascaris equorum Oxyuris equi, Strongyloides westeri cestoda Anoplocepha spp.,*	Acid ether technique, Flotation concentration Cellophane tape methods	92 fecal	29.35%, 19.56%, 8.7%, 5.43%, 4.35%, 3.26%.	Erbil	Zangana et al., 2013
Horses		Microfilaria (*Setaria spp.)*	Knott's technique	78	30.76%	Mosul	Suleiman et al.,2020

Risk factors of endo and ectoparasites in equids

Several studies in Iraq have shown differences in hard tick infestation rates and their relationship to monthly frequency. The prevalence of infestation of hard ticks increases during rainy seasons and decreases during dry seasons and this is linked to the necessity for tick eggs to have a high humidity rate for hatching, which may reach 60% (Estrada-Pea et al., 2006). The number of mites decreases in the summer and grows in the winter owing to changes in temperature and humidity, which impact the mite's spread, the rate of egg laying and growth, and the mite's reproductive activity, which increases in the fall and is more active in the winter (Littlewood, 2011). In Iraq, the largest percentage of equid infestation was observed in April, while the lowest infection rate was recorded in November, with no infestation reported in December, January, or February (Aziz and AL-barwary, 2019; Faraj, 2013). This explains why the environmental parameters, such as humidity and temperature, are insufficient for tick reproduction and movement. These variances might be related to variations in environmental parameters, such as rainfall, temperature, and relative humidity. Erbil

province is situated between mountains and hills, as well as plains. The northern parts are mountainous, the eastern areas are semi-mountainous with hills, and the western sections are hilly, but the southern areas are flat. Furthermore, there are differences in the rates of infestation across various locales. The North zone of Erbil province had the highest rate of hard tick infestation, while the West zone had the lowest rate. There were no statistical differences between the East and South zones (Aziz and AL-barwary, 2019). *Boophilus annulatus* was the most common species among equids in this study in Iraq's Erbil provinces (Aziz and AL-barwary, 2019). Furthermore, Shuber et al. (2014) and Mohammed (2015) observed *B. annulatus* infestation rates of 12.5% and 16.7% in horses and donkeys in central Iraq, respectively. This might be because the majority of horses were transported from Baghdad and central Iraq to the province of Erbil. In Iraq, the greatest occurrence of *Sarcopties sacbiei* mange in horses was in November, with 10%, and the lowest incidence was in January, with no infection recorded in September, October, April, or May (Faraj, 2013). Equine piroplasmosis (EP) susceptibility is influenced by a variety of parameters, including the type of equid, gender, age, breed, health state, origin, activity, pregnancy, seasons, management, and the presence of ticks on the animal (Garca-Bocanegra et al., 2013; Sumbria et al., 2016). (Aziz and AL-Barwary, 2019) said that there were no significant variations in the types of equids, gender, or age categories, indicating that EP is common in Iraq's Erbil governorate. According to Alsaad et al. (2012), the seroprevalence of *Babesia caballi* and *Theileria equi* was much greater in horses than in donkeys, and vice versa. Furthermore, infection rates were substantially greater in females than in males according to gender (Sray et al., 2019; Al-Rammahi et al., 2020). Female horses appeared to be infected with microfilariae at a greater rate than male horses (Hadi and Atiyah, 2014). The rationale is that stress-induced immune suppression during the third trimester of pregnancy and parturition may be the outcome of greater protozoan infections in female equids, particularly if continuously infected, increasing their risk of disease exposure (Afridi et al., 2017). Significant age differences were found between infected horses with EP, with equines under 2 years old being more infected than equines over 2 years old (Al-Rammahi et al., 2020). Prochno et al., 2014 and Faraj et al., 2019 concluded that the prevalence of *T. equi* and *B. caballi* in all ages of equids was the same. Theileria equi levels were significantly higher in recreational equines than in racing equines (Aziz and AL-barwary, 2019; Sray et al., 2019).This might be due to a physical stressor that temporarily suppresses the immune system, and animals with impaired immune systems have been proven to be more vulnerable to infection (Sevinc et al., 2008). Significant differences in horse theileriosis were also observed across geographical locations. This difference might be attributed to differences in the distribution of the disease vector (tick), as well as the availability of ideal weather that aided in the multiplication of intermediate vectors (Al-Rammahi et al., 2020). When equids infected with ticks were compared to equids not infested with ticks, the prevalence of *T. equi* was significantly higher. Furthermore, when equids maintained with other animals were compared to equids kept alone in the stable, the seroprevalence of EP was two times greater (Aziz and AL-barwary, 2019). In general, geographical areas within and across governorates in Iraq had a substantial impact on the seroprevalence of *T. equi*, *B. caballi*, and both protozoa infections (Sray et al., 2019; Al-Rammahi et al., 2020). Management techniques, host activity, sample size, the existence of competent tick vectors, and other meteorological conditions such as temperature, humidity, and rainfall that affect tick habitat (Kouam et al., 2010, Garca-Bocanegra et al., 2013), may all have a role in seroprevalence. In addition, as compared to May, the seroprevalence of EP was significantly higher in July, November, and December (Aziz and AL-barwary, 2019). Also,

Golynski et al., 2008, who found that EP prevalence is influenced by seasonal factors. These results, however, contradict those of Moretti et al. (2010), who reported that the season had no effect on the incidence of EP infection. The prevalence of the genus *Eimeria* in draught horses in Iraq varied significantly during the months. April, May, and June had the greatest prevalence, while January and December had the lowest (Kalef, 2015). Within the group of draught horses infected with *Eimeria* spp., there was no statistically significant difference in prevalence between males and females. Furthermore, the seroprevalence of *Eimeria* spp. was considerably greater in draught horses and Fourosia club horses at the ages of 2-4 and 4-6 years (Kalef, 2015). Furthermore, male horses were infected with *Cryptosporidium* spp. and *Giardia* spp. at a much higher rate than female horses.In Iraq, Cryptosporidium infection is very common in both humans and animals (Alali et al., 2021b). While the age of the horse has no effect on the infection rate with these protozoa, the prevalence of *Cryptosporidium* spp. and *Giardia* spp. in horses has been shown to vary significantly across geographical areas (Butty, 2011; Altaee et al., 2014). In contrast, Moosa, 2019 stated that the infection rate of Cryptosporidium spp. was substantially greater in foals than in adults. Furthermore, infection with equine worm eggs is common in the north and center of Iraq, may be owing to the presence of equines (horses and mules) in these regions' communities and mountains (Hadi, 2011). In the case of equine helminth, there were significant differences in the percentages of infections in horses and donkeys of various ages, but no significant differences in the percentages of infections in males and females of horses and donkeys (Wannas, 2012). According to Zhangana et al. (2013), the prevalence rate of infection was higher in young horses under the age of 5 years than in older horses.

Treatment of Equids

Parasites should be controlled permanently to reduce the effect it on equids. Most of drugs are traditional and used in treatment of adults and foals. A single dose of ivermectin 0.2 mg/kg BW as an oral paste was shown to be more effective than a single dose of oxibendazole 10 mg/kg BW as an oral solution in treating draught horses with gastrointestinal and lung worms (Al-Saad and Abdul-Majeed, 2006). At 14 days after treatment, a mixture of ivermectin and closantel was 100% effective in eradicating eggs of *P. equorm* and *O. equi*, and 99.42% for *Strongylus* spp, as well as larvae of *Gastrophilus nasalis* (Albadrani and Aldelami, 2009). Pyantel, tetrahydropyrimidines, benzimidazoles, and macrocyclic lactones may have been used to control horse nematodes.

Antihelmintic resistance in horse cyathostomins and *Parascaris equorum* may develop as a result of improper usage of anthelmintics (Hodgkinson, 2006; Raza et al., 2019). There are a variety of medications available to treat EP, each with varying degrees of efficacy. Some pharmaceuticals, such as oxytetracycline hydrochloride, tetracycline, and anti-theilerial compounds such as parvaquone and buparvaquone, are known to be less successful for treating *T. equi* (AL-Mola and Al-Saad, 2006). When administered at two doses of 4 mg/kg body weight IM within 48 hours, imidocarb dipropionate was shown to be effective in the treatment of EP infection in horses, with treated animals recovering entirely, appearing normal and clinical signs returning to normal on the sixth day (AL-Mola and Al-Saad, 2006). In addition, a supportive therapy for EP treated with imidocarb dipropionate may include aspirin at a dosage of 10 mg/kg of body weight IM, repeated after 48 and 72 hours, and heparin at a dose of 100 IU/kg of body weight subcutaneously, repeated after 48 and 72 hours (Alsaad and Mohammad, 2011).

DISCUSSION

Parasitic diseases infect a variety of hosts and are found throughout the world, causing significant economic losses. The variation in prevalence rates might be attributable to sample size differences and environmental factors that impact both parasites and vectors. These findings could be due to the high number of ticks in Iraq and the equids' constant exposure to infected ticks. Equine piroplasmosis (EP) is a tick-borne disease that causes fever, jaundice, petechiation, depression, anemia, dyspnea, sweating, conjunctivitis, decreased appetite, colic, and sudden death in horses. It is caused by two hemoprotozoal parasites (*Theileria equi* and *Babesia caballi*) (Zhao et al., 2020). Table 1 depicts the frequency of infection (up to 100%) and the diverse group of parasites that affect it, particularly hemoparasites. Helminthes (roundworms and flatworms) infecting ruminants, horses, and birds may infect domestic and wild animals (Raue et al., 2017; Matthews and Burden, 2013; Hamzah, 2020). Many factors, both direct and indirect, might have an impact on young animals. Because several major helminth parasites that infect donkeys also infect horses, animals that co-graze may be a source of infection for both helminthes. Infection of the gastrointestinal tract is known to be acquired passively via the eating of infective larvae on pasture (Hadi, 2011). The apicomplixan parasites (*C. parvum, E. lukarti,* and *G. intestinalis*) are zoonotic gastrointestinal protozoans that are linked with diarrhea, colic, and lack of appetite. Different parasites may infect all grazing animals, and certain animals may infect all humans and animals in the same region (Altaee et al., 2014). Donkeys, on the other hand, co-graze with horses and have permission to highly transmit to others. The flukes, *Fasciola hepatica* and *Dictyocaulus arnifildi* may also infect horses. This flatworm may be passed from ruminants to equids through snails on grass. In Iraq, ticks were the most prevalent ectoparasite isolated from horses. Ticks are non-permanent, obligatory ectoparasites of vertebrates and the most common ectoparasites of vertebrates, posing a severe hazard to human and animal health (Ueti et al., 2008; Al-Moula and Rahemo, 2004; Shubber et al., 2014). Infections varied significantly according to epidemiological risk variables (gender, age, location, activity, and season). Adequate feed supply and limiting prolonged open grazing of donkeys and horses are critical. Overworking/overloading, poor husbandry practices, general negative attitudes toward this species, and limited veterinary care programs are all risk factors (Camino et al., 2019; Sray et al., 2019). Microscopical diagnosis is unreliable, particularly during chronic infection of equids with equine piroplasmosis, whereas serological surveys may be useful in detecting infections but are not specific, and using modern methods, such as molecular and nanotechnology, which are more specific, for species identification (Sray et al.,2019). Chemical medications are more prevalent in Iraq, and resistance to anthelminthic pharmaceuticals may develop. These animals are mostly utilized for sport clubs, transportation systems, raising awareness among animal owners, and providing adequate deworming and preventives to lessen the parasites' economic impact (Matthews and Burden, 2013).

CONCLUSION

The frequency of parasitic illnesses in equids in Iraq was found to be quite high in this study. Due to the huge number of different horse parasites documented in recent literature reviews, as well as the few studies completed in Iraq, further research is needed. The variability in parasite prevalence rates in these studies can be attributed to a number of factors, including the analysis techniques used, sample size, farmers' lack of use of anthelminthics, and feeding horses on pastures contaminated with infected eggs or third stage, which can cause re infection and remain in the ground, while food and pastures persistently become sources of infection. These findings stimulate the development of further diagnostic

techniques for detecting all *T. equi* and *B. caballi* genotypes in other animals in order to reduce the danger of importing carrier equines into Iraq. Finally, parasitic illnesses and infestations of equines are common in Iraq and are caused by a wide range of parasites (protozoa, helminthes, ectoparasites, and parasitic flies) that endanger the health and wellbeing of the animals. In order to effectively manage the parasites, further study is required in Iraq to uncover new species, chronic infections, and infection risk factors.

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Three-Dimensional Printing Applications in Veterinary Surgery

ABSTRACT

Thanks to three-dimensional (3D) image structuring methods, 3D printing products have been used for many purposes in veterinary medicine in recent years. It can be used in many stages like vocational training in veterinary surgery, informing the patient before the operation, surgery planning, surgical method rehearsal, patient-specific intraoperative drilling and cutting guide, patient-specific implant, prosthesis, or orthosis production. The fact that the patient-specific model can be produced with 3D printing and its similarity to reality, the economic and minimal microbial risk makes 3D models attractive. It is inevitable that its effective use will become widespread in Turkey with its advantages such as the advantages it provides in treatment, being economical and allowing patient-specific procedures. In this article, the potential of the use of 3D printing products in veterinary medicine and especially in veterinary surgery, the stages of 3D printing production, current applications, areas of use, current situation, and future are examined in detail. Thanks to the 3D model, the physiopathology and treatment process can be shown more clearly on the organ model to patient owners, providing great convenience to veterinarians. Veterinarians can produce any material that they can use in clinical practice with 3D printing. Apart from these basic applications, advanced surgical planning and rehearsal procedures, production and intraoperative use of patient-specific drilling and cutting guides, production of patient-specific implants and various biomaterials, and other applications that have been studied have effective advantages in increasing the success of treatment. In case the surgical method requires a complex series of procedures and the area to be operated includes complex and intricate structures, the success of the surgery is increased by performing advanced surgical planning with 3D printing products. Thanks to this rehearsal, shortening the operation and anesthesia time, reducing the possibility of mistake and iatrogenic damage in the surgical procedure, pre-planning the materials and implants to be used according to this model, and bending the implants if necessary, giving the ideal shape before the operation provide important advantages. It is inevitable that 3D printing will be used more widely and effectively in veterinary surgery in the near future. Studies on the use of 3D printing technology in veterinary clinical sciences, especially in veterinary surgery, will provide significant benefits and original contributions to veterinary surgery practice.

Keywords: Additive manufacturing, dog, cat, animal, three-dimensional printing, 3D

INTRODUCTION

The use of new technologies in human and veterinary medicine carries the diagnosis and treatment practices one step further. Many innovations are emerging and developing in veterinary surgery such as imaging methods, diagnostic devices, surgical rehearsal models, drilling and cutting guides, new implants and biomaterials. Along with these, the treatment expectations of animal owners are also increasing. The use of current technologies by veterinarians in treatment processes is undoubtedly one of the most important factors in increasing success.

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Review Article

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3D printing technology enables solid products to be obtained by adding two-dimensional micron-level layers and without applying other processes such as cutting and drilling. This technology, which Physics engineer Dr. Charles Hull invented in 1984, started to become widespread in 2006 (Emre et al., 2015). While it was a very expensive technology in the beginning, its prices decreased day by day and its spread continued. With 3D printing technology, products can be produced in many areas and with various materials. Materials such as polylactic acid (PLA), polyamide (PA, nylon), polycarbonates (PC), acrylonitrile butadiene styrene (ABS) can be used as raw materials in 3D printing products that will not be implanted into the organism (Hespel, 2015). Some biopolymers, bioglass and bioceramics, metals and alloys can be used as 3D printing raw materials in medical treatment applications (Arslan et al., 2018; Emre et al., 2015). It is possible to use more than one material and color. In addition, a wide variety of cellular materials can be used in the production of artificial tissues and organs as materials in bioprinters (Jamieson et al., 2021).

In recent years, 3D printing products have been used for different purposes, thanks to methods that allow 3D image configuration such as computed tomography (CT) and magnetic resonance (MR) imaging, which are increasingly used in veterinary medicine. It can be used for undergraduate and graduate education in basic sciences such as anatomy and physiology, and at many stages in clinical sciences, both in professional education and treatment processes. It can be used in many stages like vocational training in veterinary surgery, informing the patient owner before the operation, surgery planning, surgical method trial, patient-specific intraoperative drilling and cutting guide, patient-specific implant, prosthesis, or orthosis production. Although these areas of use are not yet routinized in the world, they attract a lot of attention and are spreading rapidly. It is inevitable that its use will become widespread also in Turkey with its advantages such as the features it provides in treatment, being economical, and allowing patient-specific procedures. In this article, the potential of the use of 3D printing products in veterinary medicine and especially in veterinary surgery, the stages of 3D printing production, current applications, areas of use, current studies, and the future in the world and in Turkey are examined in detail.

3D PRINTING in VETERINARY MEDICINE

Models produced with 3D printing in veterinary medicine are more attractive than cadavers and other ready-made organ models. For this reason, it has primarily been used in undergraduate and graduate training, workshops, and courses. Being able to produce patient-specific and closest to reality models with 3D printing, being very economical, and having minimal microbial risk are important advantages. Through the 3D model, explaining the pathophysiology and treatment process of the disease on the organ model to patient owners in a more understandable way also provides great convenience to veterinarians. In addition, veterinarians can design any material or part that they can use in clinical practice (such as laryngoscope, IV suspension apparatus, patient positioning materials in X-ray) in 3D and produce them with 3D printing. Apart from these basic applications, advanced surgical planning and trial procedures, production and intraoperative use of patient-specific drilling and cutting guides, production of patient-specific implants and various biomaterials, and other applications that have been studied have effective advantages in increasing the success of treatment (Bose et al., 2019; Hespel et al., 2014). Standard implants may be inadequate in areas such as the skull (with its anatomically special shape) that restrict the surgeon, in cases with

large defects, or in cases such as anomalies. At this point, patient-specific implants and biomaterials have a vital function (Altuğ et al., 2015; Hespel, 2015). Printers that can produce tissue or organs (with living cells) are also called 3D bioprinters. Promising results are obtained regarding 3D printing products that are biocompatible and that can be used in tissue or organ replacement, and studies are continuing intensively (Jamieson et al., 2021). There are also studies on the production of pharmaceutical products to be used in veterinary medicine with 3D printing (Martin et al., 2020; Sjöholm et al., 2020). The first study on the clinical use of 3D printing products with 3D image reconstruction in veterinary medicine was published by Harrysson et al. (2003). In the following years, the work continued increasingly. The use of 3D printing continues to develop for different purposes at many stages from diagnosis to treatment.

The Benefits and Advantages of 3D Printing Products in Veterinary Surgery

- Providing a more detailed and realistic understanding in diagnostic procedures
- Making the most realistic training applications on the 3D model economically
- More effective and accurate explanation of diagnosis and treatment to patient owners
- Surgical planning and consultation on the 3D model in more detail, advanced, and close to reality
- Trial of the patient-specific surgical procedure on the 3D model
- Reducing the duration of surgery and anesthesia, in this way reducing other risks that may arise if prolonged
- Minimizing risks such as iatrogenic damage and blood loss in surgeries
- Implementation of the planned method with the least margin of error thanks to the 3D printed patient-specific surgical drilling and cutting guides
- Depending on all these, increasing the success and effectiveness of surgical treatments and reducing complications

How to 3D Print for Veterinary Surgical Purpose?

Simple 3D product designs can be prepared by scanning the material to be produced with 3D scanners, making the desired changes on the 3D scan image or designing it directly on the computer software. For the patient-specific 3D models, radiological images suitable for 3D reconstruction such as CT or MR imaging should be taken. CT or MR scans of each patient are taken under general anesthesia by placing the appropriate position. In these scans, the section thickness is (~0.625-2 mm) planned in accordance with the detail precision, animal type, and size of the model to be produced. The 3D image reconstruction or rendering process, which means converting two-dimensional CT or MR images into 3D images, is created in the virtual environment in DICOM (Digital Imaging and Communication in Medicine) file format with computer software. On this created virtual 3D model, the desired shape can be given to the 3D printing by performing the desired manual retouching, adding (designing a drilling/cutting guide on it, designing an implant, etc.) and isolation of certain parts (endotracheal tube, a tissue layer, etc.). This 3D model is then converted to STL (Surface Tesellation Language) format, which is a CAD (Computer Aided Design) compatible file format (Hespel et al., 2014; Li et al., 2018; Oxley, 2017; Winer et al., 2017). The reconstructed 3D image in the ready STL file format is transferred to the 3D printer and the necessary slicing is done on the software for 3D printing. The product is prepared for printing by determining the raw material suitable for the purpose. Following all the details are completed, the 3D printing process starts. The product obtained at the end of the process is

kept in water or a solution suitable for the material in order to dissolve the filling material that fills the gaps in the 3D printed product. Thus, the organ model takes its final form, which is ready for use with the opening of the cavities.

Usage Areas of 3D Printing in Veterinary Surgery

In case the surgical method requires a complex series of procedures and the area to be operated includes anatomically or pathologically complex and intricate structures, the success of the surgery is increased by performing advanced surgical planning with 3D printing products. In addition, by rehearsing the surgical procedure, shortening the operation and anesthesia time, reducing the possibility of error in the surgical procedure, pre-planning the materials and implants to be used according to this 3D model, and if necessary, bending the ideal shape before the surgery provides very important advantages (Bose et al., 2019; Hamilton-Bennett et al., 2018; Hespel, 2018; Worth et al., 2019).

The use of patient-specific produced surgical drilling or cutting guides in rehearsal and real surgeries increases the success and effectiveness of surgeries and reduces the risk of complications. This is an important advantage especially in brain and neurosurgery (Figure 7 and 8), sensitive surgeries in areas containing nerve tissues in and around the skull and spine, and corrective or reconstructive surgery where the organ takes a highly abnormal form (such as angular deformities) due to disease. In orthopedics and traumatology, 3D printing products are most needed for fractures in irregular bones (such as skull), craniomaxillofacial surgeries, comminuted fractures, damage with tissue loss, anomalies, and developmental disorders. Since minor errors can cause irreversible damage in neurosurgery, especially during internal fixation procedures to be applied to the vertebrae, surgical rehearsal on a 3D model which is exactly the same as the patient's related vertebrae, and the intraoperative use of 3D printed patient-specific drilling guides have opened a new era in neurosurgery (Hamilton-Bennett et al., 2018; Hespel, 2018; Toni et al., 2020). Non-metallic 3D printing products can be produced quite economically, but it is costly to manufacture patient-specific implants from metal materials such as titanium. For this reason, patient-specific 3D printing implant production has not yet become as widespread in veterinary surgery as in humans. However, in the future, this may become widespread as it becomes more economical.

Benefiting from the advantages of 3D printing products increases the success in soft tissue surgery, in operations in regions with complex anatomical structures such as the cardiovascular system (Figure 1), in transplantation surgeries, or in the removal of tumoral masses shaped adjacent organs with important vital functions.

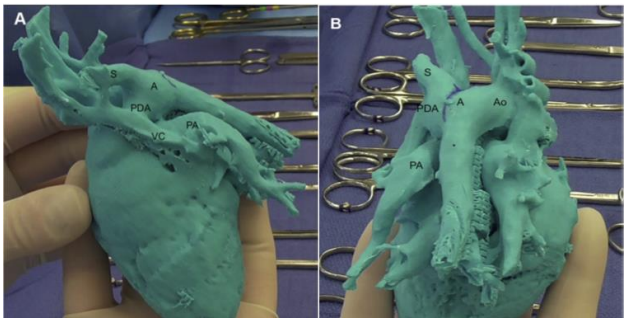

Figure 1. Left lateral (A) and dorsal (B) view of a 3D-printed full-scale model using CT angiography in a dog with multiple cardiovascular anomalies. A: aneurysmal enlargement; PDA: patent ductus arteriosus; S: abnormal left subclavian artery; PA: left pulmonary artery; Ao: right aortic arch; VC: vena cava (Dundie et al. 2017).

The development of 3D bioprinter products, which are planned to be implanted in the body in humans and animals, is also an issue that scientists work on. It is aimed to achieve great advances in the treatment of many surgical diseases by examining 3D bioprinter products with in vivo studies in experimental animals after in vitro research and developing them in accordance with the desired properties (Bose et al., 2019; Jamieson et al., 2021; Popov et al., 2019).

Advanced Preoperative Planning and Surgery Trial

Harrysson et al. (2003) were able to physically examine the deformities on the pre-surgical 3D printed model of a German Shepherd with multifocal deformities in the hind limbs and rehearsed advanced surgical planning and osteotomy. In this way, they stated that the use of 3D printing products is definitely more beneficial than two-dimensional evaluations or 3D images that are not printed, with their advantages such as performing the most accurate surgical technique, shortening the operation time and obtaining the advantages related to this, and therefore better clinical results (Harrysson et al., 2003). Dismukes et al. (2008) performed advanced surgical planning and rehearsal with 3D printing for angular deformity surgery in a dog and reported that it was very useful and beneficial. Crosse and Worth (2010) performed advanced surgical planning and osteotomy rehearsal with a 3D-printed prototype before the corrective surgery of an angular deformity in a dog and suggested that successful treatment with a single-stage corrective osteotomy is possible thanks to the 3D-printed prototype. In the surgical treatment of angular limb deformities, which is common in dogs, 3D printing products can be used for pre-surgical advanced planning, trial, ensuring full compatibility of the implant (with applications such as plate bending and shaping), patient-specific production of cutting or drilling guides to be used in surgery, and even the production of patient-specific plates or other implants. When used for these purposes, 3D printing products increase success, reduce complications, reduce the time of surgery and anesthesia, and save costs by eliminating the related negative causes (Harrysson et al., 2015; Marcellin-Little et al., 2008). In addition, in veterinary surgery, due to the fact that there are many anatomical differences due to species or race in veterinary surgical practices and there are no standard ready-made implants produced specifically for the bones like in human medicine, it is emphasized that the need for patient-specific implant production is more than the humans (Harrysson et al., 2015). In Hespel's (2015) study, a biopsy or surgical resection method performed with a 3D-printed skull model was described in dogs with cranial masses. In a horse where multi-part fractures in the orbit, periorbital wall and temporomandibular joint occurred together, the most accurate surgical technique is realized by pre-surgical planning and rehearsal, thanks to the 3D printing product model (Hespel, 2015). Winer et al. (2017) published a large study (including 32 cases) in which they used 3D printing products for advanced preoperative planning in oral and maxillofacial surgeries in small animals. In this study, reconstruction after mandibulectomy in 12 dogs, reconstructive treatment of fracture nonunion complication in 6 dogs and 2 cats, ostectomy of temporomandibular joint ankylosis in 4 dogs, cleft palate repair in 2 dogs and 1 cat, neoplasms in anatomically difficult areas in 2 dogs and 1 cat were investigated. In this study, 3D printing products were used for reconstruction after mandibulectomy in 12 dogs, reconstructive surgical treatment of nonunion complications in 6 dogs and 2 cats, ostectomy of temporomandibular joint ankylosis in 4 dogs, cleft palate repair in 2 dogs and 1 cat, removal of neoplasms in anatomically difficult areas in 2 dogs and 1 cat, reconstructive surgery of traumatic anatomical disorders in 2 dogs. They state that advanced preoperative planning and surgical rehearsals with 3D printing are very useful in these applications, and 3D printing is an excellent tool in this regard (Winer et al., 2017). Van Duijl et al. (2018) performed the surgical planning procedures in a cat with osteoma in the mandible with the 3D printed model and reported its advantages. Lam and Kim (2018) used 3D computer-aided planning and 3D printing modeling for the surgery of a complex articular femur fracture in a dog. In this study, they stated that it is very useful in terms of planning, implant selection and implant bending or preparation processes and surgical rehearsal, and it is also very useful in undergraduate and specialist

(postgraduate) education. Bordelo et al. (2018) benefited from the advantages of advanced planning and osteotomy rehearsal with a preoperative 3D printed product model in a dog with an angular deformity (radius curvus), and they recommend it by stating that they found it useful. Mejia et al. (2019) compared the accuracy of the biomodels produced by 3D printing for the radius bone in dogs with real bones by making measurements and analyzes and showed that there is no significant difference, therefore they could be used in preoperative planning and orthopedic surgery. Blake et al. (2019) demonstrated the beneficial applications of 3D printing products in small animals and wild animal species in many areas from preoperative planning to surgical procedures in veterinary surgery and reported that their popularity is increasing day by day.

3D Printing in Veterinary Orthopaedics and Traumatology

Many orthopedic and traumatological pathologies such as congenital anomalies, developmental skeletal deformities, dysplasias, traumatic fractures in anatomically intricate regions in animals are the most common and difficult to treat cases in veterinary surgery (Altuğ et al., 2015). There are significant gains in overcoming these difficulties with the work done with 3D printer products. Marcellin-Little et al. (2008) stated that in the tibial plateau corrective osteotomy (TPLO) technique, thanks to the cutting guide and TPLO plates that they produced specifically for the patient with 3D printing, the technique provides better stability as well as faster and more accurate application. Crosse and Worth (2010) found that 3D printed products such as corrective surgical limb models or cutting guides are beneficial in providing more accurate treatment in dogs. Kuipers von Lande et al. (2012) achieved successful results in a dog in which they applied rapid prototyping with 3D printing technology and permanent hard palate defect repair with a patient-specific produced titanium plate. Petazzoni and Nicetto (2014) produced a patient-specific plate with the 3D printed prototype they produced for pancarpal arthrodesis surgery in a dog and stated that the results were quite successful. Another study has shown a 3D printed patient-specific titanium alloy prosthetic implant was successfully used in a dog with a large defect in the radius bone (Harrysson et al., 2015). In a study, the stem of a total hip replacement implant placed in the femur in dogs was produced by 3D printing and tested in vitro (Marcellin-Little et al., 2010). 3D printing was used to prepare a patient-specific full knee replacement in a dog with irreparable loss of bone tissue due to a gunshot injury, and for producing a humeral head implant in another dog (Liska et al., 2007; Sparrow et al., 2014). Castilho et al. (2017) produced tricalcium phosphate (TCP) cages with 3D printing to be used as an implant in tibial tuberosity advancement (TTA) surgery of cranial cruciate ligament ruptures in dogs and achieved successful results. Lee et al. (2017) applied the bone grafts they produced using PCL/TCP composite material with 3D printing together with stem cell therapy in the treatment of maxillary bone defects in dogs and achieved successful results. Oxley (2017) stated that the 3D printed patient-specific cutting (osteotomy) and drilling guides for bilateral shoulder arthrodesis surgery in a dog are very useful, effective in the reduction and optimal implant placement, and also reduce the surgical time. In another study by Oxley (2018), a 3D printed patient-specific guide was successfully used for the treatment with the minimally invasive plate osteosynthesis (MIPO) technique of a comminuted humeral fracture (Figure 5) in a cat. Park et al. (2018) produced PCL/TCP composite bone grafts with 3D printing and used them successfully in experimental canine maxillary defects (Figure 2). Kim et al. (2018) were used a 3D printed patient-specific graft in the repair of a large maxillary bone defect caused by tumor resection in a dog, and they found the results to be successful.

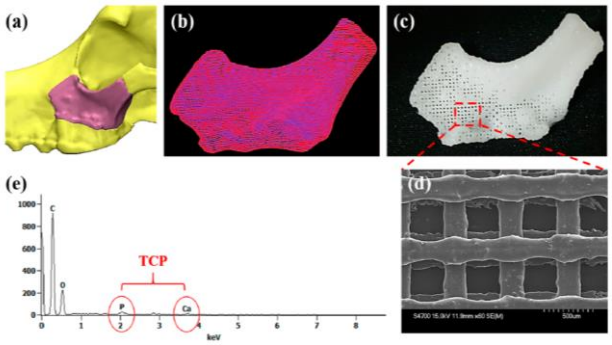

Figure 2. The 3D printing process of PCL/TCP composite bone grafts used in experimental canine maxilla defects: (a) 3D configuration of the defect; (b) print design; (c) 3D printed graft, (d) its SEM image, and (e) energy emissive spectrometry results (Park et al. 2018).

Due to the irregularly shaped bones, 3D printing provides significant advantages in craniomaxillofacial applications where patient-specific implants are required in the mandible, maxilla and skull bones. In cases such as defects in this region, mandibulectomy and large oronasal fistula cases, it is possible to use exactly compatible 3D printed patient-specific implants (Harrysson et al., 2015; Schöllhorn et al., 2013). Carrel et al. (2016) used 3D printed TCP/HA bone grafts to the canine mandible as a pilot study and reported that the results were promising and that further studies should be done. Southerden and Barnes (2018) also reported that they achieved very good results with the advantages of 3D printing technology in the treatment of mandibular fracture in a cat, and excellent jaw occlusion was provided. Popov et al. (2019) reported that 3D printed patient-specific prosthetic implants for bone defects after osteosarcoma resection in dogs gave successful results. Zanfabro et al. (2021) have employed a 3D printed pre-operative surgical planning model for monolateral temporomandibular joint ankylosis treated with piezoelectric surgery in a cat.

Although studies on the use of 3D printing products in veterinary orthopedics and traumatology are limited, it is increasing day by day. In Turkey, there is no published research article on this subject yet. However, there are two different case report congress presentations, a poster and an oral presentation. These are the reports presented as the use of a 3D printed titanium alloy jaw prosthesis in a sea turtle (Caretta caretta) with irreparable defects (Figure 3) in the mandible and maxilla (Altuğ et al., 2015) and a prosthesis made of PLA material with 3D printing in an amputated dog (Sen, 2020). Therefore, the use of 3D printing in veterinary orthopedics and traumatology has begun and will develop in Turkey.

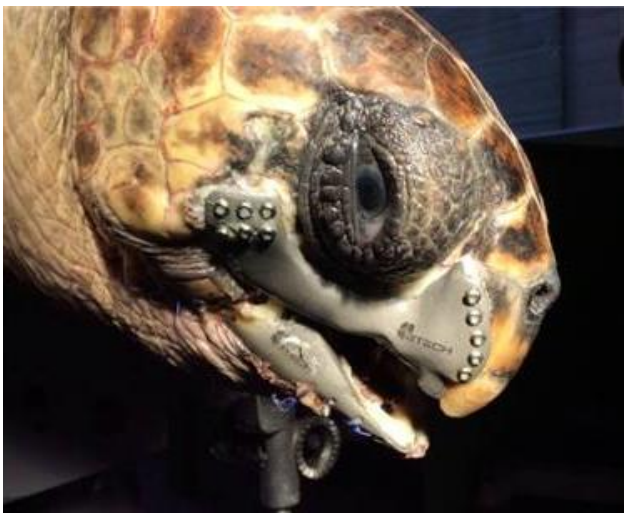

Figure 3. Postoperative image of 3D printed patient-specific titanium alloy prosthetic implants for large defects of mandible and maxilla damage in a Caretta caretta sea turtle (Altuğ et al. 2015)

3D Printing in Veterinary Neurosurgery

With the help of 3D printing, Hespel (2015) has provided detailing of the pre-surgical diagnosis, advanced surgical planning, rehearsal of the surgery, preparing the plates and screws to be applied according to the 3D model of atlantoaxial luxation in two dogs. These are important especially in terms of preventing possible iatrogenic damage (to not entering the medullary canal and preventing damage to the spinal cord). In a dog with comminuted axis vertebra fracture, the fracture was evaluated in detail and medical treatment was decided, and recovery was achieved successfully. In the research of GM2 gangliosidosis, which is a genetic disease, prototypes of the skull and cervical vertebrae of two cats were produced by 3D printing and evaluated. Bronchial structures were produced by 3D printing and used for the

purpose of training in endoscopy application in healthy cats and dogs. In addition, it was stated that many problems in communication were overcome thanks to the explanation of many pathologies through physical examples to patient owners with 3D printed models (Hespel, 2015). In spinal surgery, 3D printing products provide unique advantages when intervertebral disc prosthesis, spine stabilization, and fixation are required (Harrysson et al., 2015; Schöllhorn et al., 2013). Hamilton-Bennett et al. (2018) produced patient-specific drill guides with 3D printing for cervical transpedicular screw applications in 3 dogs and applied a total of 32 screws in 3 cases. They reported that screw applications can be successfully performed in the desired direction and placement, clinical results are improved, and surgery time and morbidities are reduced (Hamilton-Bennett et al., 2018).

Suñol et al. (2018) reported that 3D printing model production is beneficial and economical in the identification and education of vertebral fractures in dogs. The fact that studies showing that a surgeon in the field of neurosurgery can perform the same surgery in a 25-33% shorter time after 20 years of experience reveal the importance of 3D printing technology's contribution to shortening the operation times (Hespel, 2018). Years of experience have been gained by the surgeon's ability to predict more accurately the surgical manipulations and complete the procedure in a shorter time, such as examining the 3D printing product by touching, grasping the details, advanced planning before the surgery and rehearsing the surgical method. Patient-specific drill guides also provide a very important advantage by reducing the margin of error.

In one of the recent studies, Gutmann et al. (2020) demonstrated with measurements and analyzes that they are more successful than other standard brain biopsy tools used for the same purpose as the stereotactic brain biopsy guides they produced for dogs with 3D printing (Figure 6). Successful applications with these guides, being suitable for skulls of all sizes, and high millimetric accuracy in the positioning of brain biopsy needles are important results. Toni et al. (2020) produced patient-specific drilling guides with 3D printing for pedicle screw application in the lumbosacral region in dogs and showed that the placement directions of the screws are more accurate and safe for use in surgery. They state that patient-specific drill guides produced with 3D printing are safe, effective, and successful (Toni et al., 2020). De Armond et al. (2021) developed a special surgical guide system for bipolar coxofemoral osteochondral allograft transplantation in dogs with 3D printing and tested it on canine cadavers. They reported that thanks to this special guide, the surgical time is reduced and the accuracy of the surgical procedure is improved (De Armond et al., 2021).

3D Printing in Soft Tissue Surgery

The prevalence of diseases requiring surgery in organs with delicate and anatomically intricate structures such as eyes and ears is substantial (Altuğ and Deveci, 2016; Deveci et al., 2020; İşler et al., 2015). Dundie et al. (2017) produced a full-scale 3D print of the heart and vessels before the surgical procedure of a patent ductus arteriosus (PDA) case with 5 different cardiothoracic vascular anomalies in a 10-week-old dog, with significant advantages in terms of detailing the diagnosis, full evaluation, advanced surgical planning, intraoperative communication, and coordination. In this study, it was emphasized that thanks to the 3D model, the anesthesia and surgery times were shorter, and it was especially beneficial in providing atraumatic vascular dissection in surgery (Dundie et al., 2017). Soares et al. (2018) achieved successful results in a cat with chronic oronasal fistula by applying a 3D printed patient-specific soft tissue flap (mesh). In another study, the bronchial structures of a Savanah monitor lizard and a Nile crocodile were 3D printed (Hespel, 2015). Nibblett et al. (2017) produced a canine external ear canal model with 3D printing for use in otoscopy training and stated that the

same application can be made on this model with a real dog. Dorbandt et al. (2017) used 3D printing to take advantage of advanced surgical planning and other advantages in the treatment of orbital and periorbital masses in three dogs. While discussing its advantages, they stated that revolutionary developments can be achieved in veterinary ocular surgery with the use of 3D printing (Dorbandt et al., 2017).

The Future of 3D Printing in Veterinary Surgery

There is no workshop, course, or published research article on this subject in Turkey, either in veterinary surgery or other veterinary clinical sciences. There are only two papers mentioned above (Altuğ et al., 2015; Sen, 2020). Therefore, the studies carried out in this field are studies of the extremely high original value. The ability to obtain 3D bioprinted products with the development of 3D printer technology is a groundbreaking advance in human and veterinary surgery applications. 3D bioprinting differs from traditional 3D printing. In order to allow for the generation of complex functional tissues, it utilizes bioinks comprised of cells and other biomaterials. It has found success in human medicine studies both in vitro and in vivo. Recent efforts investigated its veterinary application and continue. To date, it has been produced cardiovascular, cartilage, bone, corneal and neural constructs in animal species, by 3D bioprinting. Moreover, the use of animal-derived cells or models in human research has provided additional information for veterinary translation (Jamieson et al., 2021).

Shim et al. (2014) produced a bone regeneration membrane with 3D printing to be used in experimental calvarial defects in rabbits (Figure 4) and obtained promising results.

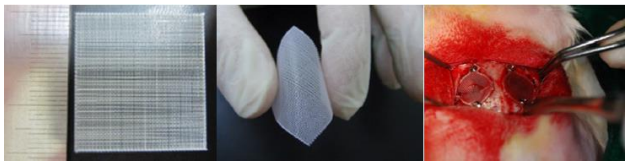

Figure 4. Preoperative and intraoperative images of 3D printed bone regeneration membrane containing PCL/PLGA/β-TCP fixed with screws for bilateral full-thickness calvarial defect repair in an in vivo experimental study in rabbits (Shim et al. 2014).

Li et al. (2014) produced polyethylene lung prostheses with 3D printing to prevent displacement of the mediastinum in order to prevent late postpneumectomy complications in their experimental research on dogs. Irradiation has been used to sterilize these 3D bioprinter products. Dogs which underwent pneumectomy with a 3D printed prosthesis were successful, results in significantly fewer complications after one-year follow-up compared to dogs without prostheses (Li et al., 2014). Many unsolved problems have been solved with studies using 3D printing in human and veterinary surgery, and significant progress is being made with ongoing studies (Akbaş et al., 2018). Promisingly, the production of tissues and organs with 3D bioprinting is a field of study that has been intensely researched and progressed in recent years. The beginning and progress of the use of 3D printing studies carried out in the world in both clinical and experimental veterinary studies in Turkey will also make significant contributions to comparative scientific studies in human medical sciences. Witowski et al. (2017) stated that the models produced with 3D printing are more beneficial than 3D image reconstruction, and they emphasize that studies on this subject in clinical and surgical fields are very necessary. Harrysson et al. (2015) report that in their research on the use of 3D printing technology in veterinary surgery, superior advantages have been obtained with diagnosis, surgical training, advanced planning and rehearsal before surgery, and patient-specific implant and biomaterial production.

Scientific studies around the world on the use of 3D printing in the field of veterinary surgery are summarized above. It is seen that this technology, which has become widespread day by day in various countries of the world, has been used in veterinary clinical sciences, positive

and effective results have been obtained, recommended, and still developing by continuing to be studied intensively. It is obvious that its use will be much more common in the future (Carrel et al., 2016; Demirkan et al., 2018; Dorbandt et al., 2017; Hespel, 2018; Hespel et al., 2014; Kinns et al., 2011).

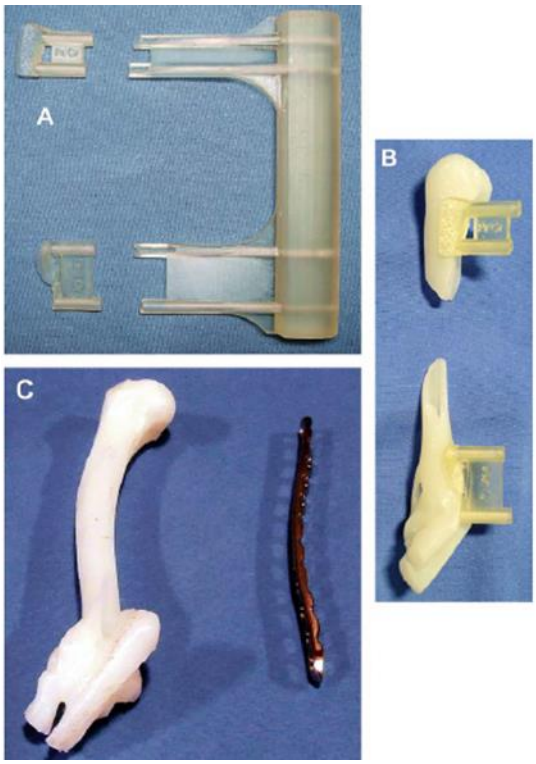

Figure 5. A: Two Ellis pin routing guides and reduction guides produced by 3D printing; B: View of Ellis pin routing guides superimposed on proximal and distal bone fragments produced by 3D printing. C: 3D printed bone mold by modeling the opposite intact humerus bone in the other limb, and the plate shaped with this mold before surgery (Oxley 2018).

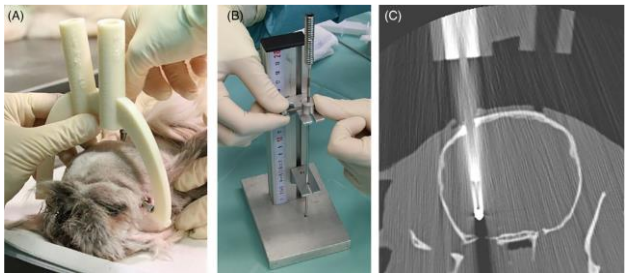

Figure 6. A: Patient-specific 3D-printed brain biopsy guide in a dog. B: Adjusting the depth of the biopsy needle. C: Transversal CT image with a biopsy needle (Gutmann et al. 2020).

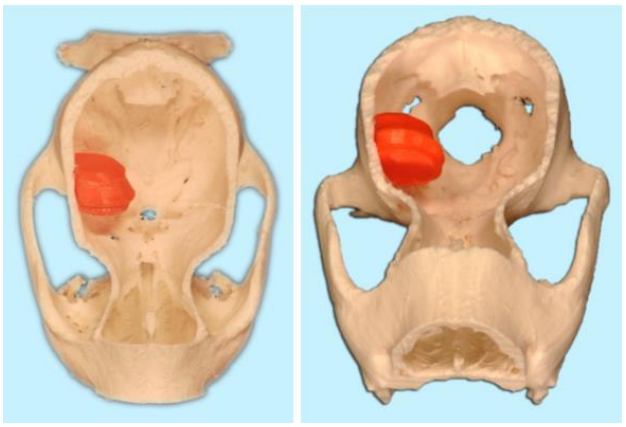

Figure 7. Dorsoventral (a) and oblique (b) view of a 3D printed model of an open cranium with a brain tumor (Hespel et al. 2014).

Figure 8. Dorsoventral view of a 3D printed model of cervical vertebrae with atlanto-axial luxation. On this 3D printing model, the appropriate shaping of the plates by bending and screw applications was rehearsed (Hespel et al. 2014).

CONCLUSION

In veterinary surgery, unique and important advantages are provided by the use of 3D printing technology at all stages from diagnosis to treatment. It is inevitable that 3D printing will be used more widely and effectively in veterinary surgery in the future. While developments in the use of 3D printing technology continue rapidly in both human and veterinary medicine applications around the world, its use in veterinary clinical sciences continues as a fairly new field for various reasons in Turkey, despite the fact that studies on this subject have started and progress has been made in human medicine. Studies on the use of 3D printing technology in veterinary clinical

sciences, especially in veterinary surgery, should be encouraged and supported as they will provide significant benefits and original scientific contributions to veterinary surgery practice in Turkey.

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