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Contents

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Comparison of Carcass Weight and Carcass Characteristics in Some Cattle Breeds

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ABSTRACT

Beef is an indispensable source of protein for humans. The production is increased in recent years with the effect of increasing population. Besides of beef cattle breeds, male calves of dairy breeding and combined productive animals are also been fattened. The aim of the study investigate the carcass yield and characteristics of the some beef cattles. In the study hot carcass weights, carcass characteristics and skin weights of 588 male Holstein, Brown-Swiss, Simmental and Montbeliarde cattle breeds raised in Amasya region were used. For this purpose, the hot carcass weight data was taken at the slaughterhouse shortly after dressing and the weights of mince, cubed meat, steak, tenderloin, ribeye at the chilled carcass and skin weights of the beef cattle raised by the members of the Amasya Cattle Breeders Association were investigated. Slaughtered animals were fed mainly concentrated feed with commercial fattening feed. The hot carcass weights of slaughtered animals in Holstein, Brown-Swiss, Simmental and Montbeliarde cattle breeds were 343.70±3.12 kg, 319.80±15.50 kg, 336.56±3.58 kg and 349.44±6.06 kg respectively. The hot carcass weights of the animals according to age of 13-15 months, 16-18 months, 19-21 months, 22-24 months and +24 months were 316.44±8.20, 337.44±3.18, 342.00±3.62, 339.98±5.48 and 370.35±9.11 kg respectively. While the effect of breed on hot carcass weight was insignificant ($p>0.05$), the effect of age was significant ($p<0.05$). The breed and age interaction were significant ($p<0.05$). The highest skin weight was determined in the Simmental breed, and the lowest in the Holstein breed. While the effect of breed on mince, cubed meat, steak, tenderloin fillet was insignificant ($p>0.05$) and it was significant on ribeye ($p<0.05$). However, while the effect of age on mince, cubed meat, steak, tenderloin was significant ($p<0.05$), it was not significant on ribeye ($p>0.05$). As a result of the study, it was evaluated that different breeds reached their target slaughter weights in different times, and that breeders determine the target slaughter time according to carcass yield rather than age.

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INTRODUCTION

Animal originated food play a big role in human nutrition. They contain protein, vitamins and minerals that are essential for human. The digestibility of these nutrients is also quite high (Ardıçlı, 2018). Animal foods include meat, milk and eggs. For the continuity of human life, the production of animal foods must continue without interruption.

The male calves of dairy and combined yielded cows are also fattened besides of beef calves in Türkiye. These breeds include Limousin, Charolais, Angus, Hereford, Holstein, Montofon, Simmental and Montbeliard. Artificial insemination undoubtedly plays a major role in raising superior quality breeding animals. In this way, breeding program has been successful throughout the country (Kayar and İnal, 2019; Şenyüz et al., 2020; Kayar and İnal, 2022; Fidancı et al., 2022). Average beef carcass meat has reached the average of developed countries in Türkiye. Average carcass weight ofcattle were 218.20 kg in the world, 267.90 kg in European countries, 284.50 kg in Türkiye and 311.40 kg in America (FAO, 2022).

In order to obtain optimum carcass yield in cattle, beef or combined yield animals must reach sufficient maturity. Carcass yield varies depending on breed. Fattening period is between 15-18 months. This period is suitable for economical meat production (Ergün et al. 2020). However, this period may take longer due to various reasons in terms of animal husbandry. Likewise, the carcass is expected to have a certain weight in order to be evaluated economically. While the bone ratio is high in the carcasses of animals that have not completed their maturation, the fat ratio increases in the carcasses of animals that are subjected to excessive fattening. This situation affects the evaluation of the carcass. The skin, another animal product obtained during slaughter, is varies depending on the breed and age of the animal. In the literature, carcass weights were 251.1 - 320.0 kg in Holstein steers, 293.3 - 344.4 kg in Simmental steers, 268.2 - 289.4 kg in Brown-Swiss steers, and 297.4 - 343.2 kg in Montbeliarde steers (Alberti et al., 2008; Çatıkkaş and Koç, 2017; Nikoloau et al., 2020; Sipahi et al., 2022).

Shredding of beef carcasses is generally done according to the valuable meat classification (Alpan, 1972; Sarıözkan et al. 2013). However, there is not much evaluation in terms of final consumer use. In this study, hot carcass yields, carcass characteristics and skin weight were examined which obtained from beef cattle slaughtered in the Amasya Region. The aim of the study investigate the carcass yield and characteristics of the Holstein, Brown-Swiss, Simmental and Montbeliarde beef cattles.

MATERIAL AND METHOD

In the research data obtained from animals after slaughtering in the slaughterhouse in Amasya. For this purpose, 588 male cattle were examined which slaughtered between 2018 and 2021. Amasya Breeding Cattle Breeders Association (ABCBA) slaughter data was used. The breeds of the cattle were Holstein (n = 268), Brown-Swiss (n = 18), Simmental (n = 231) and Montbeliarde (n = 71). The animals were fattened to intensive in different enterprises and fed with concentrated feed based. The breed and age information of the animals was checked from the Breeding Association records.

After the animals were slaughtered in the slaughterhouse, their hot carcass weight and skin weights were determined via electronic scale (0.1 kg sensitivity) and recorded. After this stage, the carcasses were kept in +4 C° cold storage for 24 hours and cooled to +4 C°. Chilled carcasses were stripped from the bones and divided into minced, cubed meat, steak, tenderloin and ribeye in processing plant of ABCBA according to the Alpan (1972). Each separated portion of meat was weighed and recorded.

The data were analyzed with Anova multivaried comparison test in the SPSS (2001) package program, and in case of a difference between the groups, the Tukey test was applied. The significance level was determined as 0.05.

RESULTS

The hot carcass weights and standard errors in the study were presented in Table 1. There were no difference was between breeds in terms of hot carcass weight ($p>0.05$). The effect of breed was significant in terms of skin weights ($p<0.05$). While the effect of breed was insignificant in terms of minced, cubes meat, steak and tenderloin obtained by deboning after cooling the carcass ($p>0.05$), it was significant in terms of ribeye ($p<0.05$). The hot carcass weights, skin weights, chilled carcass weights of minced, cubes meat, steak, tenderloin and ribeye of the animals according to their slaughter ages were given in Table 2. The hot carcass weight was significant according to slaughter ages ($p<0.05$). Likewise, a significant difference was found between skin weights according to age ($p<0.05$). While minced, cubed meat, steak and tenderloin obtained after deboning the chilled carcass was significant according to age ($p<0.05$), ribeye was insignificant ($p>0.05$). Breed*age interaction was significant in all parameters ($p<0.05$).

Table 1. Carcass, skin and carcass charasteristic weights of cattle breeds.

Breed	n	Carcass Weight (Kg)	Skin Weight (Kg)	Mince Weight (Kg)	%	Cubed Meat Weight (Kg)	%	Steak Weight (Kg)	%	Tenderloin Weight (Kg)	%	Ribeye Weight (Kg)	%
Holstein	268	343,70±3,12	42,70±0,42^{b}	152,99±2,50	44.51	73,41±3,37	21.36	8,00±0,14	2.33	3,99±0,06	1.16	9,33±0,12^{a}	2.72
Brown-Swiss	18	319,80±15,50	41,94±1,74^{b}	168,10±0,40	52.56	59,16±2,96	18.50	7,55±0,60	2.36	4,09±0,44	1.28	7,68±0,72^{b}	2.40
Simmental	231	336,56±3,58	46,81±0,44^{a}	152,13±2,77	45.20	77,85±2,64	23.13	8,50±0,13	2.53	4,15±0,07	1.23	8,95±0,14^{ab}	2.66
Montbeliarde	71	349,44±6,06	44,67±1,14^{ab}	157,38±4,90	45.04	74,42±7,94	21.30	8,28±0,42	2.37	4,25±0,14	1.22	9,00±0,29^{ab}	2.58
p		0.72	0.001	0.57		0.26		0.65		0.20		0.03	

Table 2. Carcass, skin and carcass charasteristic weights of cattle breeds according to age.

Age (Month)	n	Carcass Weight (Kg)	Skin Weight (Kg)	Mince Weight (Kg)	%	Cubed Meat Weight (Kg)	%	Steak Weight (Kg)	%	Tenderloin Weight (Kg)	%	Ribeye Weight (Kg)	%
13-15	38	316,44±8,20^{c}	40,78±1,67^{c}	135,36±7,48^{b}	42.78	65,28±8,08^{b}	20.63	7,07±0,45^{b}	2.23	3,70±0,22^{b}	1.17	8,33±0,54	2.63
16-18	176	337,44±3,18^{bc}	43,50±0,48^{c}	153,47±2,88^{ab}	45.48	64,84±4,86^{b}	19.22	7,76±0,15^{ab}	2.30	3,92±0,08^{ab}	1.16	9,19±0,14	2.72
19-21	175	342,00±3,62^{b}	43,86±0,73^{bc}	160,22±2,60^{a}	46.85	77,88±3,62^{ab}	22.77	8,29±0,18^{a}	2.42	4,12±0,08^{ab}	1.21	9,28±0,17	2.71
22-24	83	339,98±5,48^{bc}	46,47±0,57^{ab}	155,90±4,36^{ab}	45.86	66,84±2,88^{ab}	19.66	8,45±0,23^{a}	2.49	4,18±0,12^{ab}	1.23	9,36±0,25	2.75
24+	66	370,35±9,11^{a}	46,89±0,73^{a}	157,56±5,71^{ab}	42.54	82,83±3,88^{a}	22.37	8,26±0,20^{ab}	2.23	4,26±0,10^{b}	1.15	9,24±0,23	2.50
p		0.001	0.001	0.03		0.01		0.01		0.02		0.15	

DISCUSSION

In the study, it was observed that the carcass weights of Holstein, Brown-Swiss, Simmental and Montbeliarde were similar. In studies conducted on the Holstein breed, hot carcass weight was reported by Alberti et al., (2008) as 320.0 kg; by Golebiewski and Brzozowski (2011) as 293.7 kg; by Catikkaş and Koç (2017) as 304.36 kg; by Nikolaou et al., (2020) as 251.1 kg and Sipahi et al., (2022) as 264.9 kg. The values in the current study were higher than the literature. However, it was lower than reported by Kim et al., (2021) (442.9 kg). Hot carcass weight value in the Brown-Swiss was higher than determined by Alpan (1972), Kızıl and Aydoğan (2014), Çatıkkaş and Koç (2017), Pınarbaşı and Yazgan (2020) and Sipahi et al., (2022). The data obtained in the current study on the Simmental breed were higher than those Çatıkkaş and Koç (2017), Duru and Sak (2017), Nikolaou et al., (2020), Gao et al., (2022) and Sipahi et al., (2022) and similar with Alberti et al., (2008) and Pınarbaşı and Yazgan (2020), lower than those reported by Kızıl and Aydoğan (2014), Şenyüz et al., (2020) and Ateş and Akbaş (2022). The value obtained in the Montbeliarde breed was higher than reported by Sipahi et al., (2022), similar with by Nikolaou et al., (2020) and Golebiewski and Brzozowski (2011), it was lower than the values reported by Chládek, and Žižlavský (2004) and Chládek, and Žižlavský (2005). It is thought that the difference in hot carcass weights is related to slaughter age and feeding management. It is also important in genetic structure. In fact, it is known that some genes play an important role in parameters that directly affect carcass yield, fat storage, body weight, and body length in cattle (Daş, 2016).

The highest hot carcass weights were obtained from +24 months of age animals. The lowest hot carcass weight was at the age of 13-15 months of age of animals. The values obtained in the current study were, regardless of race, higher than Alberti et al., (2013), Duru and Sak (2017), Özdemir and Yanar (2021) and Sipahi et al., (2022) according to slaughter age. Also similar with Alberti ve ark. (2008) and lower than those of Chládek and Žižlavský (2004), Chládek and Žižlavský (2005) and Kim et al., (2021). The optimum slaughter age was 18 months for beef cattles (Ergün et al., 2020). The findings obtained in the study was resemble with the literature. Carcass yield was low at the slaughter time before the optimum slaughter age, and higher after slaughtering. This situation was expected. However, considering the amount of consumed feed for the carcass yield, it didnot appear to be an advantageous situation. Because, although feed and dry matter consumption increased with age in beef cattle, live weight does not increase at the same rate (Hayırlı, 2022).

There was a significant difference between breeds in terms of skin weight. The highest skin weight was 46.81±0.44 kg in the Simmental, and the lowest was 41.94±1.74 kg in the Brown-Swiss. In the literature, the skin weights reported by Alpan (1972) and Duru and Sak (2017) in Brown-Swiss, Holstein and Simmental were lower than the current study, while the values reported by Kızıl and Aydoğan (2014) and Ateş and Akbaş (2022) were higher. When compared to skin weights obtained in the study were lower in Brown-Swiss and Holstein breeds than the beef cattles, Simmental and Montbeliarde were similar with the Limousin, Charolais, Angus and Hereford (Kayar and İnal, 2022). In cattle, skin weight was directly proportional to body surface area and therefore live weight. It is thought that the difference in skin weights was due to the live weights of the animals at slaughter.

There was no difference between breeds in terms of minced, cubed meat, steak and tenderloin weights. However, ribeye weight was higher in the Holstein than the Brown-Swiss. Depending on the age of cattles, the situation was the opposite. While minced, cubes, steak and tenderloin weights were significant in depending on the age, but it was similar in terms of ribeye weight. There are not many studies on the evaluation of meat. In the literature (Alpan, 1972; Arpacık, 1978), the weight of tenderloin

and steak was lower than the current study, and the minced weight was higher. In a study conducted on the Zavot cattle (Sarıözkan et al., 2013), tenderloin values was similar with the study, but steak and tenderloin values were higher. Especially the rate of valuable meats, the effect of breed and age is important.

CONCLUSION

As a result, the fattening performance of the male steers of the combined breeds was above world standards in the study. The Montbeliarde has the potential to be higher in terms of carcass weights between the breeds. In terms of slaughter age, although the carcass weights of animals slaughtered over 24 months were higher, it was observed that the carcass weights of animals slaughtered at 19-21 months of age were at ideal weights. As a result of the study, it was concluded that breeders determine the slaughter time when they reached the target carcass weight, regardless of breed and age of cattle.

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Ethical approval
Study data were evaluated based on slaughterhouse data. No live animal data was used in the study. The authors declare that an ethics committee decision is not required.

Conflict of Interest
Author declares that there is no conflict of interest. All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Okan Oyan, Hasan Hüseyin Şenyüz and Cem Çağdaş Arköse. The first draft of the manuscript was written by Hasan Hüseyin Şenyüz and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Additional Information's
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REFERENCES

Ardicli, S. (2018). Genetik ve postmortem mekanizmaların sığır eti renk özellikleri üzerine etkisi. *Uludağ Üniversitesi Veteriner Fakültesi Dergisi,* 37(1), 49-59. https://doi.org/10.30782/uluvfd.398947

Albertí, P., Panea, B., Sañudo, et al. (2008). Live weight, body size and carcass characteristics of young bulls of fifteen European breeds. *Livestock Science,* 114(1), 19-30. https://doi.org/10.1016/j.livsci.2007.04.010

Albertí, P., Gómez, I., Mendizabal, J. A., et al. (2013). Effect of whole linseed and rumen-protected conjugated linoleic acid enriched diets on feedlot performance, carcass characteristics, and adipose tissue development in young Holstein bulls. *Meat Science,* 94(2), 208-214. http://dx.doi.org/10.1016/j.meatsci.2013.01.015

Arpacik, R. (1978). Sığır karkaslarından elde edilen etin kaliteye göre sınıflandırılması. *Ankara Üniversitesi Veteriner Fakültesi Dergisi, 25*(01).

Alpan, O. (1972). Esmer, holştayn ve simental erkek danalarında besi kabiliyeti ve karkas özellikleri. *Ankara Üniversitesi Veteriner Fakültesi Dergisi,* 19(03).

Ateş, C., Akbaş, A.A. some slaughter and carcass traits of simmental cattle slaughtered in Kırıkkale

province. *Dicle Üniversitesi Veteriner Fakültesi Dergisi,* 15(1), 37-40. https://doi.org/10.47027/duvetfd.1093049

Chládek, G., Žižlavský, J. (2004). Comparison of meat performance of Montbeliarde and Czech Pied bulls fattened to live weight of 680 kg. *Acta Univ. Agric. et Silvic. Mendel. Brun,* 52, 107-114.

Chladek, G., Žižlavský, J., Šubrt, J. (2005). A comparison of carcass proportions in Czech Pied and Montbeliarde bulls with a high carcass weight. *Czech J. Anim. Sci,* 50(3), 109-115.

Çatikkaş, E., Koç, A. (2017). Fattening performance, carcass characteristics and beef quality of Holstein–Friesian, Brown–Swiss and Simmental bulls. *Adnan Menderes Üniversitesi Ziraat Fakültesi Dergisi,* 14(1), 59-64. https://doi.org/10.25308/aduziraat.296928

Daş, A. (2016). Leptin, ghrelin ve IGF-1 gen polimorfizmlerinin etçi sığır ırklarında besi performansı, bazı karkas özellikleri ve et kalitesi üzerine etkisi. Doktora Tezi, Atatürk üniversitesi, Fen Bilimleri Enstitüsü, Erzurum.

Duru, S., & Sak, H. (2017). Türkiye'de besiye alınan simmental, aberdeen angus, hereford, limousin ve charolais irkı sığırların besi performansı ve karkas özellikleri. *Turkish Journal of Agriculture: Food Science and Technology.* 5(11), 1383-1388 https://doi.org/10.24925/turjaf.v5i11.1383-1388.1485

Ergün, A., Tuncer, Ş. D., Çolpan, İ., Yalçın, S., Yıldız, G., Küçükersan, M. K., Küçükersan, S., Şehu, A., & Saçaklı, P. (2001). Hayvan Besleme Ve Beslenme Hastalıkları. Medipress, Ankara.

FAO. (2022) Fao Statistics. https://www.fao.org/statistics/en/

Fidancı, M., Sarıözkan, S., & İşcan, K. (2022). Farklı ülkelerden ithal edilen Şarole, Limuzin ve Hereford ırkı sığırların teknik ve ekonomik açıdan karşılaştırılması. *Eurasian Journal of Veterinary Sciences,* 38(2), 75-82. https://doi.org/10.15312/EurasianJVetSci.2022.367

Gao, L., Yan, X., Liu, Y., & Xia, C. (2022). Effect of enzyme and probiotic supplementation on growth performance, nutrient digestibility, carcass traits, and meat quality of Simmental steers. *Revista Brasileira de Zootecnia,* 51. https://doi.org/10.37496/rbz5120220034

Golebiewski, M., & Brzozowski, P. (2011). Comparison of meat performance of fattening bulls and culled cows of Montbeliarde and Polish Holstein-Friesian breeds and their influence on income value from their sale. *Acta Scientiarum Polonorum. Zootechnica,* 10(3).

Hayırlı, A. (2022). *Besi Sığırlarının Beslenmesi*. 7. Ulusal ve 3. Uluslararası Sürü Sağlığı ve Yönetimi Kongresi. 20-23 Ekim 2022, Antalya. 42-48.

Kayar, T., İnal, Ş. (2019). Comparison of fattening performance of Limousine, Charolais, Angus and Hereford breed bulls. *Eurasian Journal of Veterinary Sciences,* 35(2), 104-108. https://doi.org/10.15312/EurasianJVetSci.2019.231

Kayar, T., İnal, Ş. (2022). Comparison of slaughter and carcass characteristics of Limousin, Charolais, Angus, and Hereford beef cattle in Turkey. *Tropical Animal Health and Production,* 54(6), 1-8. https://doi.org/10.1007/s11250-022-03349-7

Kızıl, S.H., Aydoğan, M. (2014). Evaluation of major cattle breeds in Turkey for slaughter and carcass traits using MANOVA and Multidimensional Scaling technique. *Erciyes Üniversitesi Veteriner Fakültesi Dergisi,* 11(1), 15-22.

Kim, S. I., Park, S., Myung, J. H., Jo, Y. M., Choi, C. B., Jung, K. K. (2021). Effect of fattening period on growth performance, carcass characteristics, and economic traits of Holstein steers. *Journal of Animal Science and Technology,* 63(5), 1008. https://doi.org/10.5187/jast.2021.e84

Nikolaou, K., Koutsouli, P., Bizelis, I. (2020). Evaluation of Greek cattle carcass characteristics (carcass weight and age of slaughter) based on SEUROP classification system. *Foods*, 9(12), 1764. doi:10.3390/foods9121764

Özdemir, V.F., Yanar, M. (2021). Effects of age at feedlot entry on performance, carcass characteristics, and beef qualitytraits of Holstein Friesian bulls reared in high altitude of Eastern Turkey. *Turkish Journal of Veterinary & Animal Sciences, 45*(5), 936-943. https://doi.org/10.3906/vet-2011-66

Pınarbaşı, A., Yazgan, K. (2020). Şanlıurfa ilinde besiye alınmış farklı sığır ırklarının besi performanslarının ve karkas özelliklerinin karşılaştırılması. *Harran Tarım ve Gıda Bilimleri Dergisi, 24*(2), 212-221. https://doi.org/10.29050/harranziraat.669266

Sarıözkan, S., Akçay, A., Bayram, D. (2013). Zavot ırkı sığırlarda karkas özellikleri ve karkas parçalamanın ekonomik yönü. *Ankara Üniv Vet Fak Derg, 60*, 257-262

Sipahi, C., Akın, A. C., Akbaş, A. A. (2022). Sığır eti arzının sıcak karkas verileri üzerinden değerlendirilmesi: Burdur ili örneği. *Eurasian Journal of Veterinary Sciences, 38*(1), 32-40. https://doi.org/10.15312/EurasianJVetSci.2022.362

SPSS, A. (2001). SPSS 13 for Windows.

Şenyüz, H. H., Erat, S., Karslı, M. A., Soydemir, İ. (2020). Comparison of fattening performance of Angus, Charolais, Limousine and Simmental cattle imported to Turkey. *Lalahan Hayvancılık Araştırma Enstitüsü Dergisi, 60*(1), 1-4. https://doi.org/10.46897/lahaed.779712

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Histopathological Investigation of Gastrointestinal System Parasites in Storks: Cathaemasia Hians

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ABSTRACT

Intestinal parasites are commonly reported in free-ranging birds and play an important role in maintaining a healthy and balanced ecosystem and causing zoonotic diseases. The aim of this work was to reveal the presence and histological findings of digestive system parasites in storks, as well as the histopathological changes caused by C. hians isolated from storks. A total of four storks, an adult black stork and three adult white storks that were brought to the pathology laboratory were used in the study. Histopathological findings of the intestines of black and white storks were determined using hematoxylin and eosin staining. During necropsy, numerous nodules were observed in the serosa of the intestines of black stork. A parasitic infection was only observed along the oesophagus. Histopathologically, in the intestines of the black stork, degeneration, necrosis and desquamation, heterophil and mononuclear cell infiltrations, parasite sections and parasite eggs, ulcerated areas, and regeneration with fibrosis were detected. Histopathologically, no parasitic infections were found in the white storks' oesophagus or proventriculus. Degeneration, necrosis, and desquamation were observed in the intestinal epithelium of white storks. This study was the first to define histopathological changes in the intestines of storks and showed severe nodular inflammation caused by C. hians in Türkiye.

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INTRODUCTION

Storks are members of the family Ciconiidae, which is part of the order Ciconiiformes, which comprises several families. This family contains 19 species of storks divided into six genera, many of which are migratory. The white stork (Ciconia ciconia Linnaeus, 1758), which is a Palearctic migrant (Girisgin et al., 2017), is a carnivore that consumes small animals, birds, amphibians, fish, and insects. The white stork lives in agricultural areas and is mostly associated with wetlands (Tylkowska et al., 2019). The black stork (Ciconia nigra Linnaeus, 1758), an endangered species of migratory waterbird (Karaska et al., 2002; Königova et al., 2015), feeds on frogs, salamanders, amphibians, fish, and mollusks found in swamps and slow-moving waters (Merino et al., 2001; Liptovszky et al., 2012). This species is one of the protected animals in our nation. Free-ranging birds play a vital role in maintaining a healthy and balanced ecosystem (Nasiri et al., 2019). Birds in zoological collections frequently carry various gastrointestinal parasites (Penczykowski et al., 2016; Moreno Manas et al., 2019). Because high levels of stress occasionally weaken the host's immune response and confinement increases the risk of inter- and intraspecific transmission, these parasites frequently cause disease in confined birds (Carrera-Jativa et al., 2020).

Numerous pathogenic parasites negatively impact humans, domestic animals, captive birds, and wildlife (including fish, birds, terrestrial mammals, and aquatic mammals) (Otegbade and Morenikeji, 2014). Both wild and domesticated birds are commonly infected by a variety of intestinal parasites that can be found in a variety of geological settings and climatic conditions, including protozoans, trematodes, cestodes, acanthocephalans, and nematodes. Because most birds serve as primary and/or secondary hosts for some parasites, this has an impact on the health of both animals and people (Girisgin et al.,2017; Nalubamba et al., 2015; Chaudhary and Pandey, 2020).

Helminths are classified as trematodes (flukes), cestodes (tapeworms), and nematodes (roundworms) (Muller, 2009). *Cathaemasia hians*, which became a Cathaemasiidae trematode, is a known parasite of storks (Ciconiformes). However, these species is rarely reported in Europe (Liptovszky et al., 2012). The gastrointestinal tracts of the white stork (Ciconia ciconia) and the black stork (Ciconia nigra) both have typical parasites of the Cathaemasia genus (Königova et al., 2015). Such trematodes have the ability to cause permanent changes in the gastrointestinal system tissues of their target hosts. Physiological digestion is directly affected by severe trematode infections. Therefore, histopathological changes are crucial for the digestive system of bird hosts. There are few studies describing how parasite infection causes histopathological abnormalities in the gastrointestinal system of wild birds. Additionally, wild birds can typically be captured at varied post-mortem times, which makes it difficult to perform specific histopathological analyses of organs (Königova et al., 2015). An in-depth and precise diagnosis is made possible by histopathological examination of organs or tissues. An understanding of pathogen interactions and their effects on the host organism can be gained by histopathological analysis (Soltysiak et al., 2014). Histopathological changes occurring during certain parasite invasions are critical for differential diagnosis and frequently confirm the presence of parasitic infections. The objective of this study was to reveal the presence and histological findings of digestive system parasites in storks as well as the histopathological changes caused by C. hians isolated from storks.

MATERIALS AND METHODS

In this study, a total of four storks were used: an adult black stork and three adult white storks brought to the Pathology Department of the Veterinary Faculty, Hatay Mustafa Kemal University, for necropsy between 2016 and 2021. All storks were necropsied, and their digestive systems were examined macroscopically and histopathologically. After fixation of tissues (especially digestive system tissues) received from all storks in 10% buffered formalin solution. The samples were routinely treated and embedded in paraffin. Sections were cut to 5 µm thickness and stained with hematoxylin-eosin (HE) (Luna, 1968; Dörtbudak et al., 2019; Colcimen et al.,2020) and examined under a light microscope. During systemic necropsy, 32 adult trematodes were collected from the oesophagus, and 34 nematodes were collected from the abdominal and thorax cavities of the black stork, particularly the air sacs. However, macroscopically, parasites were not observed in white storks. Trematodes and nematodes were fixed in 70% alcohol. Species of parasites were determined by Işık et al. (Işık et al., 2017) in the Department of Parasitology, Faculty of Veterinary Medicine, Selcuk University. However, the lesions and histopathological changes in the gastrointestinal tissues of infected storks were the focus of this investigation.

RESULTS

The nematodes found in the air sacs and abdominal and thoracic cavities during necropsy were *D. ciconiae* (Figure 1A), whereas the trematodes found along the oesophagus were *C. hians* (Figure 1C). Numerous whitish-colored nodules of hard consistency were observed in the serosa of the intestines of black stork. Other than the intestine, no other internal organs were found to have pathological lesions during necropsy (Figure 1B). The species of trematodes observed in the oesophagus were determined to be *Cathaemasia hians* by parasitological examination (Figure 1D).

Histopathologically, in the intestines of the black stork, degeneration, necrosis, and desquamation of the epithelium were observed, as were heterophil granulocyte and mononuclear cell infiltration in the propria, as well as various parasite formations and parasite eggs in both the intestinal lumen and the propria (Figure 2A-B). The ulcerated area formed because of the trematode passage into the muscular layer from the intestinal mucosa and was regenerated with connective tissue (Figure 2C). The parasites were particularly found in the muscular layer. Furthermore, parasite eggs were detected in the propria (Figure 2D). It was observed that the nodules observed macroscopically contained adult parasites and their eggs under the microscope (Figure 2E-F). No parasitic infections were observed in the oesophagus and proventriculus of white storks (Figure 3A). The proventriculus of the black stork showed epithelial degeneration, necrosis, desquamation, and mild mononuclear cell infiltration (Figure 3B). Degeneration, necrosis, and desquamation were observed in the intestinal epithelium of white storks, as well as mild cell infiltration and fibrosis in the propria (Figure 3C-D). Histopathologically, neither adult parasites nor parasite eggs were found in the white storks.

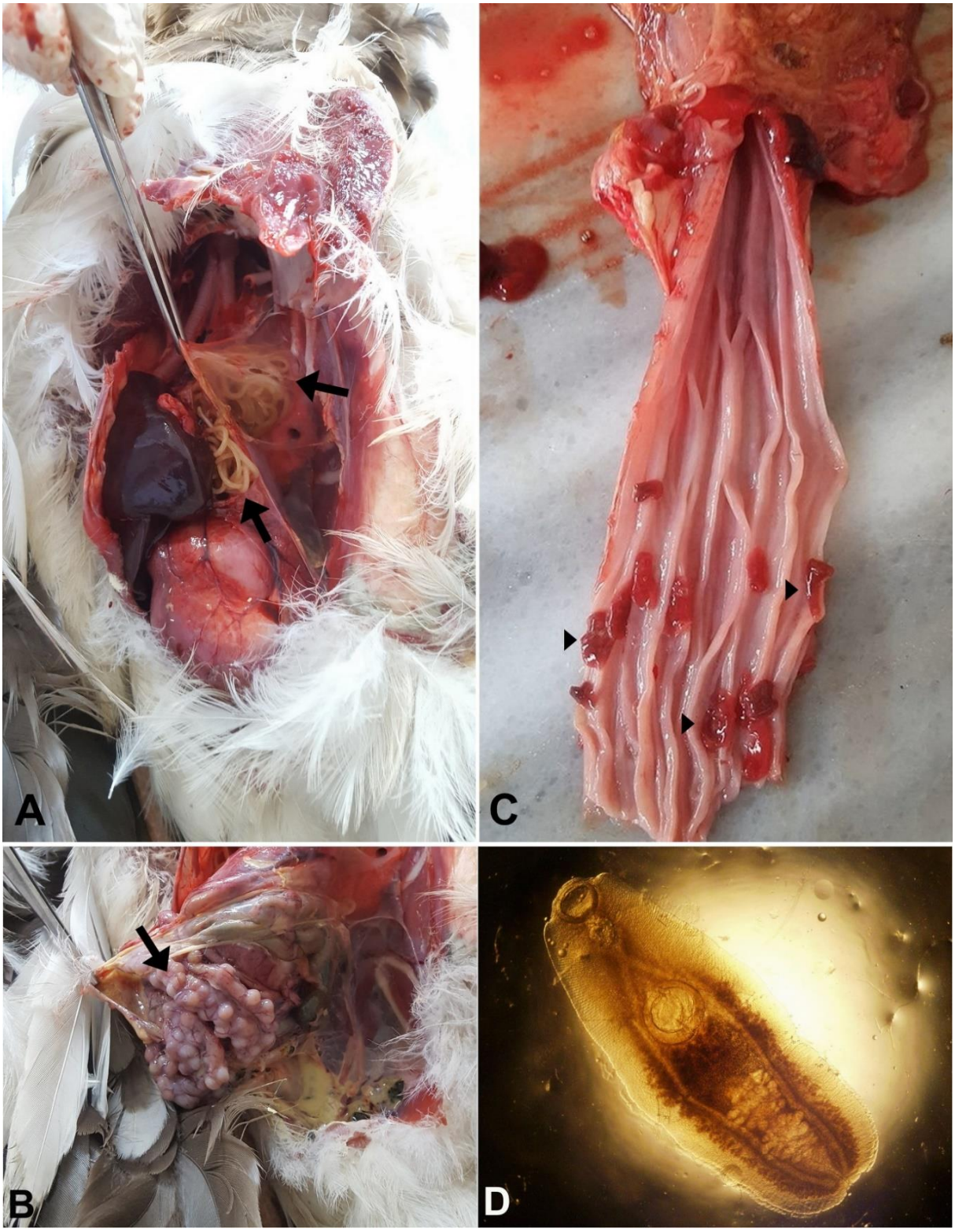

Figure 1. Macroscobic and parasitic examination. A) *Dicheilonema ciconiae* in the air sacs and thoracic cavity (arrows). B) Nodules in the intestines (arrow). C) Adult *Cathaemasia hians* in the oesophagus (arrow heads). D) *C. hians* through an optical stereoscope.

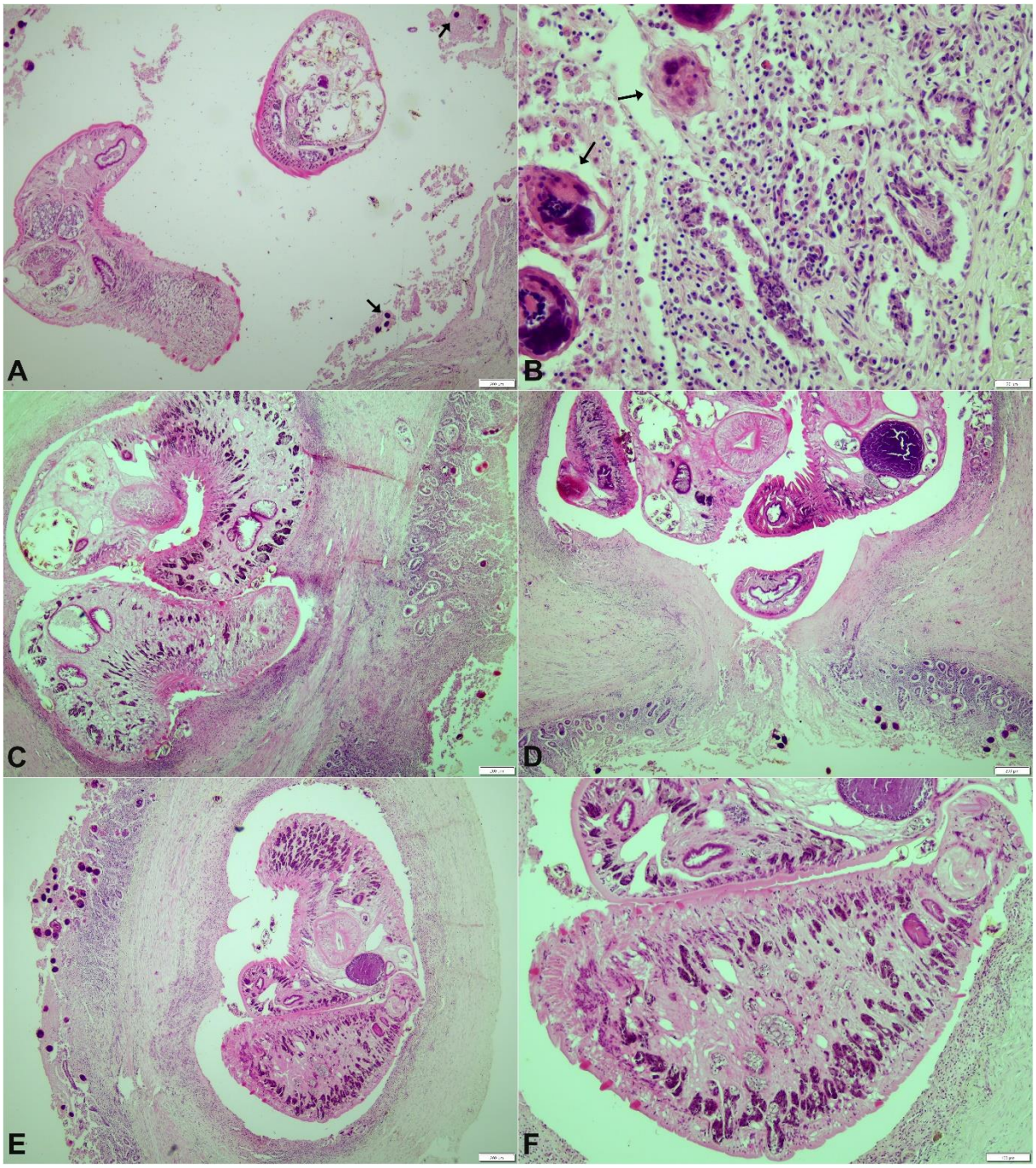

Figure 2. Microscobic findings of black stork. A) Adult trematodes and parasite eggs (arrows) in the intestinal lumen. B) Degeneration and desquamation in the epithelium, heterophil granulocyte and mononuclear cell infiltration, and parasite (arrows) in the propria. C) Regeneration with fibrosis in the mucosa and adult trematodes in the muscular layer. D) Ulcers and severity of adult and eggs of trematodes. E) Adult *C. hians* that caused nodules in the muscular layer. F) Adult *Cathaemasia hians*, HE.

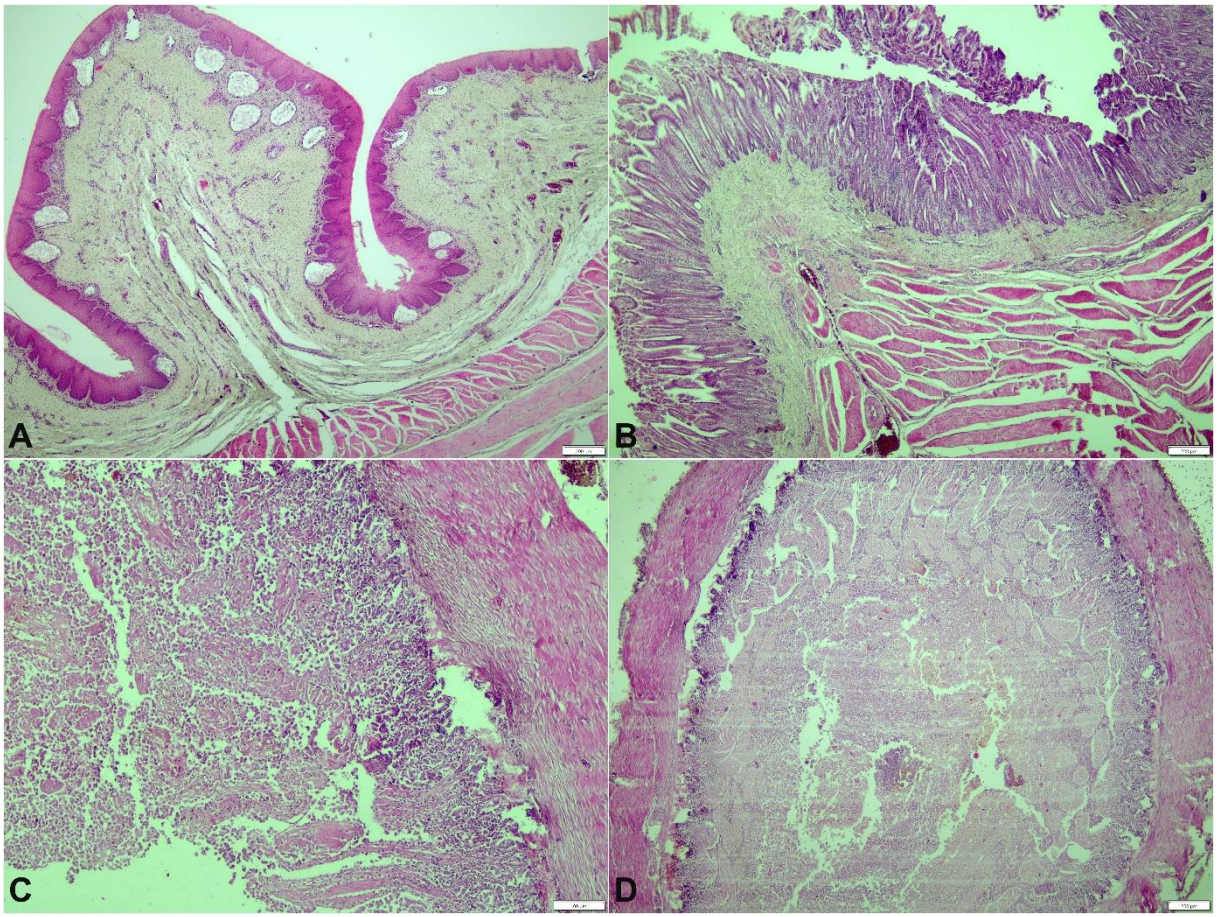

Figure 3. Microscobic findings of white storks. A) No trematode in the oesophagus. B) Degeneration and desquamation in the epithelial layer of the proventriculus. C-D) Only, degeneration, necrosis, and desquamation in the intestine and no parasites. HE.

DISCUSSION

This study focuses on adult Cathaemasia hians infection in a specific bird host, the black stork, and the histopathological changes in the infected bird's intestinal tissues. To the best of our knowledge, there are few studies on both stork and digestive system histopathology. There is a study of histopathological changes in the upper gastrointestinal system in Slovakia (Königova et al., 2015) and a study of histopathological changes in the intestine of white storks in Poland (Michalczyk et al., 2020). Moreover, infection with *C. hians* has been described in injured adult black storks and later in juvenile nesting storks (*Ciconia nigra*) in Spain (Merino et al., 2001). *C. hians* was found in a black stork after colliding with electric cables in southern Portugal (Ramilo et al., 2021). Eight species of helminths, Stephanoprora (Monilifer) spinulosa, Dictymetra discoidea, Schistocephalus solidus, Echinoparyphium sp., Chaunocephalus ferox, Tylodelphys clavata, T. excavata, and Syncuaria ciconiae, have been detected in white storks in Türkiye (Girisgin et al.,2017). The species identification of internal parasites seen in black stork in Türkiye was made for the first time by Işık et al. (Işık et al., 2017). However, to the best of our knowledge, this is the first study conducted on the histopathological findings of parasites in the intestine of storks in Türkiye.

White storks primarily feed in permanent pastures (Carrascal et al., 1993), with arthropods and earthworms (Alonso et al., 1991), furthermore, they now feed in garbage dumps near cities. On the other hand, the endangered black stork has maintained its feeding habits based on amphibia and fish, which are the most common intermediate hosts of *C. hians* (Merino et al., 2001). As the researchers stated, the

difference in trematodes obsrved in white and black storks may be related to their dietary choices. This would explain the severe nodules caused by *C. hians* in the intestine of the black stork. In addition, dietary differences may explain differences in prevalence of bird species.

Cathaemasia hians, became a Cathaemasiidae trematode, a well-known species that has members of the Ciconiidae family as definitive hosts and is commonly found in the oral cavity and occasionally in the oesophagus of these birds (Merino et al., 2001; Liptovszky et al., 2012; Ramilo et al., 2021). In the present study, macroscopically, *C. hians* was not observed in the digestive systems of white storks, whereas this trematode was found only in the oesophagus of black storks. Contrary to what the researchers reported, it was not observed in the oral cavity. Despite the presence of C. hians in the oesophagus, no change in the oesophageal tissue was observed histopathologically. It was considered that this trematode's main affinity is for the intestines. *C. hians* can cause irreversible changes in the digestive tracts of definitive hosts. Massive infections by this parasite can result in serious health problems when combined with cachexia or lowered host immunity (Königova et al., 2015). *C. hians* infections of storks have also been reported to be lethal (Sitko and Heneberg, 2015). Some researchers have reported that nodules in the intestinal walls of post-mortem storks were detected, indicating the presence of *C. hians* (Merino et al., 2001; Michalczyk et al., 2020). Furthermore, Michalczyk et al. (Michalczyk et al., 2020) have indicated that no other pathological changes or internal parasites were found except for nodular enteritis. In this study, the storks were brought in dead and without injury. While the cause of death in black storks was thought to be parasites, the cause of death in white storks had not been found. Similar to the researcher’s findings, there were numerous nodules in the serosa of the intestines and histopathologically visible ulcers caused by parasites in the intestines. In addition, trematodes were observed in the muscular layer of the intestines. Similarly, no other pathological changes were found in the intestine, except for nodular enteritis.

Dicheilonema ciconiae (Nematoda, Diplotriaenoidea) is a helminth found in birds' respiratory systems that parasitizes fish-eating birds (Syrota et al., 2016). *D. ciconiae* can be divided into two groups according to its localization type. The first group comprises parasites of organs related to the external environment that parasitize the eyes, nasal cavities, and air sacs. The second group consists of nematodes that parasitize organs that are not associated with the external environment, such as the abdominal and thoracic cavities, subcutaneous cellular tissue, muscles, joint sac, blood, and lymphatic systems (Saparov et al., 2013). *D. ciconiae* was observed in the thorax and abdominal cavity particularly in the air sacs. It is thought that it passes through the air sacs into the thorax and abdominal cavity. As stated by the researchers, it belongs to the first group and has been regarded as a parasite of organs associated with the external environment.

CONCLUSION

In this study, it was the first time that histopathological changes in the digestive system of storks in Türkiye were defined. Despite the modest number of instances in this study, it was believed that it would be more suitable to report this study, which studies alterations in the digestive systems, as a research paper because stork deaths were uncommon. The presence of trematode eggs and adults in the lumen and tissues of the stork's intestine was associated with a severe nodular inflammatory response. In the case of severe infection by parasites, tissue damage and inflammation lead to malnutrition and reduced body weight. Even severe infections can leave the host vulnerable and cause death. However, it remained unclear which parasite was the cause of death in this study, particularly in the afflicted black stork. Other infectious or poisonous factors were assumed to be the causes of death in white storks.

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Ethical Approval
This study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information, and documents presented in this study were obtained within the framework of academic and ethical rules.

Conflict of Interest
None. All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Özgür Kanat.

REFERENCES

Alonso J.C., Alonso J.A., Carrascal L.M. (1991). Habitat selection by foraging white storks, *Ciconia ciconia*, during the breeding season. *Canadian Journal of Zoology*, 69(7): 1957-1962.

Carrascal L.M., Bautista L.M., Lazaro E. (1993). Geographical variation in the density of the white stork *Ciconia ciconia* in Spain: influence of habitat structure and climate. *Biological Conservation*, 65(1): 83-87.

Carrera-Jativa P.D., Morgan E.R., Barrows M., Jimenez-Uzcategui G., Tituana J.R.A. (2020). Free-ranging avifauna as a source of generalist parasites for captive birds in zoological settings: An overview of parasite records and potential for cross-transmission. *Journal of Advanced Veterinary and Animal Research,* 7(3): 482-500. http://doi.org/10.5455/javar.2020.g445

Chaudhary B., &Pandey D.P. (2020). Snapshot view of intestinal parasites in wild birds of Chitwan District, the Southcentral Lowlands of Nepal. *Research Journal of Parasitology*, 15: 1-8. https://doi.org/10.3923/jp.2020.1.8

Colcimen N., Arihan O., Gumusok S., Kilic C.S. (2020). Effect of The Opopanax Hispidus Plant's Aerial Parts Extract on Mice Ovary. *Selcuk Medical Journal*, 36(1): 44-47.

Dörtbudak M.Y., Sağlam Y.S., Dörtbudak M.B. (2019). Sarıprenses balığında (Labidochromis caeruleus) Yoğun parazit enfestasyonuna bağlı bağırsak hasarının histopatolojik incelenmesi. *Harran Üniversitesi Veteriner Fakültesi Dergisi*, *8*(1): 52-56.

Girisgin A.O., Birlik S., Senlik B., Yildirimhan H.S. (2017). Intestinal helminths of the white stork (Ciconia ciconia Linnaeus 1758) from an inter-route site in Turkey. *Acta Veterinaria Hungarica*, 65(2): 221-233. https://doi.org/10.1556/004.2017.022

Işık N., Kanat Ö., Ceylan O., Zerek A., Güçlü F. (2017). Bir kara leylekte (Ciconia nigra) rastlanan iki helmint türü: Cathaemasia hians (Trematod, Cathaemasiidae) ve Dicheilonema ciconiae (Nematod, Diplotriaenoidea). 20. Ulusal Parazitoloji Kongresi (Uluslararası Katılımlı), 25-29 Eylül 2017, Eskişehir, 613.

Karaska D. (2002). Bocian cierny (Ciconia nigra). In: Danko S., Darolova A., Kristin A. (editors) Rozsirenie vtakov na Slovensku, Bratislava, Slovakia, 688.

Königova A., Hrckova G., Molnar L., Major P., Varady M. (2015). *Cathaemasia hians* infection in Black stork in Slovakia: morphological and histopathological study. *Helminthologia*, 52(4): 316-322.

https://doi.org/10.1515/helmin-2015-0050

Liptovszky M., Majoros G., Perge E. (2012). Cathaemasia hians in a Black Stork (Ciconia nigra) in Hungary. *Journal of Wildlife Diseases*, 48(3): 809–811.

Luna L.G. (1968). Routine staining procedures. In: Manual of histologic staining methods of the Armed Forces Institute of Pathology, McGraw-Hill Book Co NY, USA, pp. 33-46.

Merino S., Martinez J., Lanzarot P., Cano L.S., Fernandez-Garcia M., Rodriguez-Caabeiro F. (2001). Cathaemasia hians (Trematoda: Cathaemasiidae) infecting black stork nestlings (Ciconia nigra) from central Spain. *Avian Pathology*, 30(5): 559-561. https://doi.org/10.1080/03079450120078761

Michalczyk M., Sokol R., Gesek M., Mączynski M., Bedzlowicz D. (2020). Internal parasites and associated histopathological changes in deceased white storks from Poland. *Belgian Journal of Zoology*, 150: 71-80. https://doi.org/10.26496/bjz.2020.74

Moreno Manas E., Gonzalvez Juan M., Ruiz De Ybanez Carnero M.D.R., *et al*. (2019). Survey of husbandry practices for bovidae in zoos: the importance of parasite management for reintroduction programmes. *Veterinary Record*, 184(9): 282. https://doi.org/10.1136/vr.104985

Muller M.G. (2009). Parasitic diseases. In: Practical handbook of falcon husbandry and medicine, Nova Science Publishers, New York, pp. 191-222.

Nalubamba K.S., Bwalya E.C., Mudenda N.B., Munangandu H.M., Munyeme M., Squarre D. (2015). Prevalence and burden of gastrointestinal helminths in wild and domestic guineafowls (Numida meleagris) in the Southern Province of Zambia. *Asian Pacific Journal of Tropical Biomedicine*, 5(8): 663-670. http://dx.doi.org/10.1016/j.apjtb.2015.04.009

Nasiri V., Jameie F., Paykari H., Mazhari N., Soltani S., Pashaei M. (2019). Survey of gastrointestinal parasitic infection in Captive Wild Animals of a Central Zoological Garden in Iran. *Journal of Veterinary Science and Research*, 1: 78-91. https://doi.org/10.36811/jvsr.2019.110009

Otegbade A.&Morenikeji O. (2014). Gastrointestinal parasites of birds in zoological gardens in South-West Nigeria. *Tropical Biomedicine*, 31(1): 54-62.

Penczykowski R.M., Laine A.L., Koskella B. (2016). Understanding the ecology and evolution of host-parasite interactions across scales. *Evolutionary Applications*, 9(1): 37–52. https://doi.org/10.1111/eva.12294

Ramilo D.W., Caetano I., Brazio E., *et al*. (2021). Presence of one ecto- and two endoparasite species of the black stork (Ciconia nigra) in Portugal. *BMC Veterinary Research*, 17: 21. https://doi.org/10.1186/s12917-020-02724-6

Saparov K., Akramova F., Azimov D., Golovanov V., Kuchboev A. (2013). Biodiversity of filariae (Nematoda: Filariata), parasites of birds in Uzbekistan. *Turkish Journal Zoology*, *37*(6): 746-752. https://doi.org/10.3906/zoo-1106-3

Sitko J.&Heneberg P. (2015). Composition, structure and pattern of helminth assemblages associated with central European storks (Ciconiidae). *Parasitology International*, 64: 130-134. http://dx.doi.org/10.1016/j.parint.2014.11.004

Soltysiak Z., Rokicki J., Kantyka M. (2014). Histopathological diagnosis in parasitic diseases. *Annals of Parasitology*, 60(2): 127–131.

Syrota Y.Y., Kuzmin Y.I., Lyaskivskiy V.N., Kobylinsky V.V., Vasylkivska I.B. (2016). First Record of Dicheilonema Ciconiae (Nematoda, Diplotriaenoidea) From Ciconia Nigra (Aves, Ciconiidae) in Ukraine. *Vestnik zoologii*, 50(4): 379-382. https://doi.org/10.1515/vzoo-2016-0044

Tylkowska A., Stapf-Skiba A., Rzad I., Jezewski W. (2019). Helminths of white stork Ciconia ciconia (L., 1758) in north-eastern Poland. *Annals of Parasitology*, 65, Supplement 1, 45-46.

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Assessment of Role of Epizootic Hemorrhagic Disease Virus in Abortion in Cattle and Small Ruminants in Türkiye

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ABSTRACT

Epizootic hemorrhagic disease (EHD) is a vector-borne viral disease of ruminants that can cause significant economic losses due to reduced production and trade restrictions. Furthermore, epizootic hemorrhagic disease virus (EHDV) can cause abortion. However, role of EHDV in abortion in cattle and small ruminants remains uncertain. Therefore, this research aimed to assess the frequency of EHDV in aborted foetuses. In this research, a total of 2029 aborted foetuses (from 553 cattle, 1388 sheep, and 88 goats) were collected from different herds and flocks in the Aegean, Mediterranean and Central Anatolian regions of Türkiye during the period of 2012 and 2017. A real-time reverse transcription polymerase chain reaction (RT-PCR) assay was used to detect EHDV specific RNA in aborted foetuses. EHDV specific RNA was not detected within aborted foetuses. The results of the study suggest that EHDV does not play a role in abortion in cattle and small ruminants in the studied regions of Türkiye. Further research is needed to determine the role of EHDV in abortion in domestic ruminants.

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INTRODUCTION

Epizootic hemorrhagic disease (EHD), included in the notifiable diseases list of the World Organization for Animal Health (WOAH), is a vector-borne viral disease of domestic and wild ruminants, including cattle, sheep, goats, bison, mountain goats, elk, pronghorn antelope, and white-tailed and black-tailed deer (Nol et al., 2010; Favero et al., 2013; WOAH, 2021; Roug et al., 2022; Jiménez-Cabello et al., 2023).

Although EHD causes high mortality in white-tailed deer (*Odocoileus virginianus*), cattle can suffer severe disease from epizootic hemorrhagic disease virus (EHDV) infection depending on virus serotype, virulence of the strain, and host-immune status (EFSA, 2009). Sheep and goats can also be infected with EHDV, yet they do not present clinical symptoms of the disease (Mahmoud et al., 2021; Duan et al., 2022). The morbidity and mortality rates can reach 90% in white tailed deer; however, morbidity rates in cattle range from 1 to 18%, and mortality rate is lower than 1% (Yadin et al., 2008; Temizel et al., 2009).

The infectious agent, EHDV, is a non-enveloped segmented double-stranded RNA virus of the genus *Orbivirus* within the family *Sedoreoviridae*, along with African horse sickness virus and bluetongue virus (Mertens et al., 2005). It has four nonstructural proteins (NS1, NS2, NS3/NS3a, and NS4) and seven different structural proteins (VP1-VP7) (Anthony et al., 2009). Up to now, eight EHDV serotypes and two new putative serotypes have been identified based on cross neutralization tests and genetic analyses of the segment 2 nucleotide sequences of the virus (WOAH, 2021; Thabet et al, 2023). The EHDV serotypes are mainly transmitted by biological vectors, usually biting midges of the genus *Culicoides*, especially *C. obsoletus*, *C. oxystoma*, *C. imicola*, *C. mohave*, *C. brevitarsis*, and *C. sonorensis* (McGregor et al., 2019; Mendiola et al., 2019; McGregor et al., 2022; Mendiola et al., 2022; Jiménez-Cabello et al., 2023).

EHDV infection was first reported in white-tailed deer in 1955 in the United States of America (Shope et al., 1955). After that time, EHD has been reported in South and North America, Asia, the Middle East, Africa, and Türkiye (Temizel et al., 2009; Ruder et al., 2017; Qi et al., 2019; Noronha et al., 2021). In Türkiye, EHDV outbreaks caused by serotype 6 in cattle were observed in 2007 (Temizel et al., 2009). Furthermore, EHDV was detected in sheep in Türkiye (Yavru et al., 2014). A previous serological study also reported presence of EHDV specific antibodies in cattle and G*azella subgutturosa* in Türkiye (Albayrak et al., 2010).

The major clinical signs of the disease in white-tailed deer are fever, excessive salivation, swelling of the tongue, neck and head, lameness, oral ulcerative lesions, serosal haemorrhages, and sudden death (Nol et al., 2010), whereas oral ulcerations, excessive ocular and nasal secretion, swelling of the tongue, lameness, and reduced milk production are the clinical signs mostly observed in other infected ruminants (Yadin et al., 2008; Temizel et al., 2009; Savini et al., 2011; Maclachlan et al., 2015). It has been also reported that EHDV can cause abortion in ruminants (Ohashi et al., 1999; Golender et al., 2019). EHDV related abortion in cattle was reported in few studies (Golender et al., 2017; Golender et al., 2021). However, the role of EHDV in abortion in cattle and small ruminants remains uncertain. Therefore, this research aimed to assess the frequency of EHDV in aborted foetuses.

MATERIALS AND METHODS

Field Samples

In this study, a total of 2029 aborted foetuses (from 553 cattle, 1388 sheep, and 88 goats), from different herds and flocks in the Mediterranean region (Isparta, Burdur, and Antalya Provinces), Aegean region (Afyonkarahisar Province) and Central Anatolian region (Niğde, Aksaray, Karaman, and Konya Provinces), were submitted during the period of 2012 and 2017 for detection of abortifacient infectious agents to the Konya Veterinary Control Institute, were included in this study.

At necropsy, foetal tissues were collected from each foetus separately under aseptic conditions to prevent the contamination. Samples from each animal were placed into separate sterile labelled tube and were kept at -85°C until RNA extraction.

RNA Extraction from Aborted Foetuses

Foetal tissues of each foetus, weighing 20-30 mg, were placed into 2.0 ml sterile microcentrifuge tubes containing RNase and DNase free water, and were homogenized using the TissueRuptor (Qiagen, Germany). RNA was extracted from homogenates (200 µl) of foetal tissues with QIAamp Cador Pathogen Mini Kit (Qiagen, Germany), using the protocol described by manufacturer. To verify the absence of contamination, RNase and DNase free water was used during RNA extraction.

Detection of EHDV in Foetal Tissues Using Real-time RT-PCR Assay

Firstly, the extracted RNA was denatured for 5 min at 95°C, and then used for real-time RT-PCR assay. The PCR reaction mix was prepared with a RealTime ready RNA Virus Master kit (Roche Diagnostics, Indiana, USA) in a final volume of 25 µl, containing 0.12 µM of forward primer, 0.8 µM of reverse primer, 0.2 µM of probe, and 3.4 µl of sample RNA. The primers and probe used in this study are shown in Table 1. Amplification of the assay was performed using the LightCycler 2.0 Instrument (Roche Diagnostics, Indiana, USA) with the following amplification conditions: 50 °C for 8 min, 95 °C for 30 sec, and 45 cycles of 95 °C for 1 sec, 60 °C for 20 sec, and 72 °C for 1 sec. In this study, samples with cycle threshold (Ct) values < 35 were considered as positive (Maan et al., 2017).

Table 1. Primers and probe sequences used in real-time RT-PCR assay in this study

Primer	Sequence (5'-3')	Genomic target	Reference
EHDV-15-32 F	ATGTCAGCTGCGGTYTTG	Seg-9	Maan et al. (2017)
EHDV-112-85 R	TCCCAATCAACTAARTGRATYTGVATCT	Seg-9	
EHDV-69-48 P	CCTCGGTCGAACGTTGGATCAC	Seg-9	

EHDV RNA obtained from the Central Veterinary Control and Research Institute (Etlik, Ankara, Türkiye) was used as positive control, whereas RNase and DNase free water was used as negative control in real-time RT-PCR assay.

RESULTS

EHDV RNA Detection in Foetal Tissues

In the present study, 127, 87, 74, 69, 81, and 115 aborted bovine foetuses were examined in 2012, 2013, 2014, 2015, 2016, and 2017, respectively. The ages of the foetuses range from 2 to 6 months.

All tested aborted bovine foetuses were found EHDV-negative by real-time RT-PCR, and samples had Ct values ranging between 42.01 and 44.35.

In this study, 198, 217, 336, 383, 157, 97 aborted ovine foetuses were examined in 2012, 2013, 2014, 2015, 2016, and 2017, respectively. The ages of the foetuses range from 1 to 5 months. All tested aborted ovine foetuses were found EHDV-negative by real-time RT-PCR, and samples had Ct values ranging between 41.90 and 43.89.

In the current study, 3, 4, 4, 36, 27, 14 aborted caprine foetuses were examined in 2012, 2013, 2014, 2015, 2016, and 2017, respectively. The ages of the foetuses range from 1 to 5 months. All tested aborted caprine foetuses were found EHDV-negative by real-time RT-PCR, and samples had Ct values ranging between 41.79 and 44.19.

DISCUSSION

Abortions in cattle and small ruminants cause significant economic losses in the livestock industry. Mostly infectious agents (viruses, bacteria, fungi, and protozoa) cause abortion in domestic ruminants. Among the infectious agents, viruses play an important role in the occurrence of abortion and congenital malformations (Golender et al., 2017; Maclachlan and Osburn, 2017; Sick et al., 2019). Mostly, pestiviruses (border disease virus and bovine viral diarrhoea virus), bovine herpesvirus type 1, bluetongue virus, akabane virus, and Schmallenberg virus are responsible for abortions and congenital malformations in domestic ruminants (Inaba et al., 1975; Nettleton, 1990; Osburn, 1994; Graham, 2013; Endalew et al., 2019; Şevik, 2021). Furthermore, some of the EHDV field strains can also induce abortion in pregnant ruminants without clinical signs (Golender et al., 2021). The role of EHDV in abortion is still unknown. Therefore, in this research, role of EHDV in abortion cases was investigated. To the best of my knowledge, this study is the longest research that assessed role of the EHDV in abortion cases of cattle and small ruminants.

Virus isolation is a gold standard method for EHDV detection, yet it is expensive and time consuming (WOAH, 2021). Molecular diagnostic methods can detect viral nucleic acids in a short time with high sensitivity and specificity (Aslanlar et al., 2023; Görkem et al., 2020; Guclu and Ayan, 2023; Karaselek et al., 2023; Şahin et al., 2023). Furthermore, it has been reported that real time RT-PCR has high specificity and sensitivity for detection of EHDV RNA than other diagnostic methods (Viarouge et al., 2015). Therefore, in this research real time RT-PCR was used for detection of EHDV in aborted foetuses.

Until now, eight EHDV serotypes and two new putative serotypes have been identified (WOAH, 2021; Thabet et al., 2023). EHDV serotype 1 has been reported in North America, Australia, China, Egypt, Nigeria, Ecuador and Israel, serotype 2 in North America, Australia, Japan and Oman, serotype 4 in Nigeria, serotype 5 in Australia, Japan, China, Sudan, serotype 6 in North America, Australia, China, Morocco, Algeria, Libya, Türkiye, Tunisia, Oman, Sudan, French Guiana, Trinidad and Israel, serotype 7 in Australia, Japan, China, French Guiana and Israel, and serotype 8 in Italy (Ahmed et al., 2019; Yang et al., 2020; Lorusso et al., 2022; Jiménez-Cabello et al., 2023).

In this study, EHDV was not detected in the samples tested. This situation can be explained by the geographical locations of the samples used in this study. To date, only EHDV serotype 6 has been detected in Türkiye, and EHDV outbreaks were observed in cattle in Muğla Province and in sheep in Aydın Province (Temizel et al., 2009; Yavru et al., 2014). These provinces are located in western part of the Türkiye, which are suitable for reproduction of *Culicoides* spp. which are the primary vector of

EHDV. However, in this study, except Antalya Province, other provinces do not have suitable conditions for reproduction of *Culicoides* spp.

In this study, EHDV RNA was not detected in aborted foetuses. Previous studies carried out by Golender and Bumbarov (2019), Kamomae et al. (2018) in Israel and Japan respectively did not find EHDV in aborted foetuses. However, different studies from Israel reported that they detected EHDV in aborted foetuses with the detection rates ranged from 22.4 to 36.7%. (Golender et al., 2017; Golender et al., 2021). This difference in results of studies may be related to the immune status of sampled animals, the differences in farm management, the sampling strategies, and the serotype of virus. It has been reported that strains of serotype 2 and serotype 6 of EHDV can cause abortion (Ohashi et al., 1999; Golender et al., 2017). Besides, Golender et al. (2017) and Golender et al. (2021) found EHDV specific RNA only in aborted cattle foetuses, and similar to the results of this study, they could not detect the virus in aborted sheep and goats. It seems that EHDV causes abortion in cattle.

CONCLUSION

The results of the research suggest that EHDV does not play a role in abortion in cattle and small ruminants in studied regions of Türkiye. EHD mostly exists in tropical and temperate regions which support vector populations. However, genetic evolution of the virus and climate change increase the risk of introduction of the EHDV in new regions (Jiménez-Cabello et al., 2023). Additional research is needed to determine the role of EHDV in abortion in domestic ruminants.

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Ethics Approval
This research was undertaken with the permission of the General Directorate of Food and Control dated 27.12.2017 and numbered E.3335546.

Conflict of Interest
The author declares that there is no conflict of interest. Conceptualization, methodology, formal analysis, writing-review and editing by Murat ŞEVİK.

REFERENCES

Ahmed, S., Mahmoud, M.A.E., Viarouge, C., Sailleau, C., Zientara, S., & Breard, E. (2019). Presence of bluetongue and epizootic hemorrhagic disease viruses in Egypt in 2016 and 2017. *Infection, Genetics and Evolution*, 73, 221-226. https://doi.org/10.1016/j.meegid.2019.04.033.

Albayrak, H., Ozan, E., & Gur, S. (2010). A serologic investigation of epizootic hemorrhagic disease virus (EHDV) in cattle and gazella subgutturosa in Turkey. *Tropical Animal Health and Production*, 42(8), 1589-1591. https://doi.org/10.1007/s11250-010-9610-6.

Anthony, S.J., Maan, N., Maan, S., Sutton, G., Attoui, H., & Mertens, P.P. (2009). Genetic and phylogenetic analysis of the core proteins VP1, VP3, VP4, VP6 and VP7 of epizootic haemorrhagic disease virus (EHDV). *Virus Research*, 145(2), 187-99. https://doi.org/10.1016/j.virusres.2009.07.011.

Aslanlar, D.A., Aydemir, O., Kunt, M., Koc, E., & Koc, M. (2023). SARSCoV-2 VOC 202012/01(B.1.1.7) Variant: Is it More Dangerous? *Selcuk Medical Journal*, 39(1), 19-23.

Duan, Y., Yang, Z., Zhu, P., *et al.* (2022). A serologic investigation of epizootic hemorrhagic disease virus in China between 2014 and 2019. *Virologica Sinica*, 37(4), 513-520. https://doi.org/10.1016/j.virs.2022.06.005.

EFSA, (2009). Panel on Animal Health and Welfare (AHAW). *Scientific Opinion on EFSA Journal*, 7(12), 67. https://doi.org/10.2903/j.efsa.2009.1418.

Endalew, A.D., Faburay, B., Wilson, W.C., & Richt, J.A. (2019). Schmallenberg Disease-A Newly Emerged Culicoides-borne Viral Disease of Ruminants. *Viruses*, 11(11), 1065. https://doi.org/10.3390/v11111065.

Favero, C.M., Matos, A.C., Campos, F.S., *et al.* (2013). Epizootic hemorrhagic disease in brocket deer, Brazil. *Emerging Infectious Diseases*, 19(2), 346-348. https://doi.org/10.3201/eid1902.120551.

Golender, N., Khinich, Y., Gorohov, A., Abramovitz, I., & Bumbarov, V. (2017). Epizootic hemorrhagic disease virus serotype 6 outbreak in Israeli cattle in 2015. *Journal of Veterinary Diagnostic Investigation*, 29(6), 885-888. https://doi.org/10.1177/1040638717726826.

Golender, N., Khinich, Y., Bernstein, M., *et al*. (2019). Epizootic Hemorrhagic Disease Virus Type 6: Disease, RNAemia and Abortions-The Israeli Experience. *HSOA Journal of Dairy Research & Technology*, 2, 007.

Golender, N., & Bumbarov, V.Y. (2019) Detection of Epizootic Hemorrhagic Disease Virus Serotype 1, Israel. *Emerging Infectious Diseases*, 25(4), 825-827. https://doi.org/10.3201/eid2504.180149.

Golender, N., Bumbarov, V., Kovtunenko, A., *et al.* (2021). Identification and Genetic Characterization of Viral Pathogens in Ruminant Gestation Abnormalities, Israel, 2015-2019. *Viruses*, 13(11), 2136. https://doi.org/10.3390/v13112136.

Görkem, A., Uğur, A.R., Feyzioğlu, B., Özdemir, M., & Baykan, M. (2020). Çocuk Hastalarda Parainfluenza Nedenli Alt Solunum Yolu İnfeksiyonlarının Araştırılması. *Selcuk Medical Journal*, 36(2), 87-90.

Graham, D.A. (2013). Bovine herpes virus-1 (BoHV-1) in cattle-a review with emphasis on reproductive impacts and the emergence of infection in Ireland and the United Kingdom. *Irish Veterinary Journal*, 66(1), 15. https://doi.org/10.1186/2046-0481-66-15.

Guclu, E., & Cinar Ayan, I. (2023). Investigation of the Anticancer Activity of DCZ0415, a Small Molecular Inhibitor of TRIP13, in U87 Human Glioblastoma Multiforme Cells. *Selcuk Medical Journal*, 39(1), 12-18.

Inaba, Y., Kurogi, H., & Omori, T. (1975). Letter: Akabane disease: epizootic abortion, premature birth, stillbirth and congenital arthrogryposis-hydranencephaly in cattle, sheep and goats caused by Akabane virus. *Australian Veterinary Journal*, 51(12), 584-585. https://doi.org/10.1111/j.1751-0813.1975.tb09397.x.

Jiménez-Cabello, L., Utrilla-Trigo, S., Lorenzo, G., Ortego, J., & Calvo-Pinilla, E. (2023). Epizootic Hemorrhagic Disease Virus: Current Knowledge and Emerging Perspectives. *Microorganisms*, 11(5), 1339. https://doi.org/10.3390/microorganisms11051339.

Kamomae, Y., Kamomae, M., Ohta, Y., *et al*. (2018). Epizootic Hemorrhagic Disease Virus Serotype 6 Infection in Cattle, Japan, 2015. *Emerging Infectious Diseases*, 24(5), 902-905. https://doi.org/10.3201/eid2405.171859.

Karaselek, M.A., Kuccukturk, S., Kapakli, H., *et al*. (2023). Identification of the Mutation in DCLRE1C Gene by PCR-RFLP. *Selcuk Medical Journal*, 39(1), 24-28.

Lorusso, A., Cappai, S., Loi, F., *et al*. (2023). Epizootic Hemorrhagic Disease Virus Serotype 8, Italy, 2022. *Emerging Infectious Diseases*, 29(5), 1063-1065. https://doi.org/10.3201/eid2905.221773.

Maan, N.S., Maan, S., Potgieter, A.C., Wright, I.M., Belaganahalli, M., & Mertens, P.P.C. (2017). Development of Real-Time RT-PCR Assays for Detection and Typing of Epizootic Haemorrhagic Disease Virus. *Transboundary and Emerging Diseases*, 64(4), 1120-1132. https://doi.org/10.1111/tbed.12477.

Maclachlan, N.J., Zientara, S., Savini, G., & Daniels, P.W. (2015). Epizootic haemorrhagic disease. *Revue Scientifique et Technique*, 34(2), 341-351. https://doi.org/10.20506/rst.34.2.2361.

Maclachlan, N.J., & Osburn, B.I. (2017). Teratogenic bluetongue and related orbivirus infections in pregnant ruminant livestock: timing and pathogen genetics are critical. *Current Opinion in Virology*, 27, 31-35. https://doi.org/10.1016/j.coviro.2017.10.002.

Mahmoud, A., Danzetta, M.L., di Sabatino. D., *et al.* (2021). First seroprevalence investigation of epizootic haemorrhagic disease virus in Libya. *Open Veterinary Journal*, 11(2), 301-308. https://doi.org/10.5455/OVJ.2021.v11.i2.15.

McGregor, B.L., Sloyer, K.E., Sayler, K.A., et al. (2019). Field data implicating Culicoides stellifer and Culicoides venustus (Diptera: Ceratopogonidae) as vectors of epizootic hemorrhagic disease virus. *Parasites & Vectors*, 12(1), 258. https://doi.org/10.1186/s13071-019-3514-8.

McGregor, B.L., Shults, P.T., & McDermott, E.G. (2022). A Review of the Vector Status of North American Culicoides (Diptera: Ceratopogonidae) for Bluetongue Virus, Epizootic Hemorrhagic Disease Virus, and Other Arboviruses of Concern. *Current Tropical Medicine Reports*, 9(4), 130-139. https://doi.org/10.1007/s40475-022-00263-8.

Mendiola, S.Y., Mills, M.K., Maki, E., *et al.* (2019). EHDV-2 Infection Prevalence Varies in Culicoides sonorensis after Feeding on Infected White-Tailed Deer over the Course of Viremia. *Viruses*, 11(4), 371. https://doi.org/10.3390/v11040371.

Mertens, P.P.C., Maan, S., Samuel, A., & Attoui, H. (2005). Orbiviruses, Reoviridae. In: Fauquet C.M., Mayo, M.A., Maniloff, J., Desselberger, U., Ball, L.A. (editors). Virus Taxonomy Eighth Report of the International Committee on Taxonomy of Viruses. London: Elsevier/Academic Press. pp. 466-483.

Nettleton, P.F. (1990). Pestivirus infections in ruminants other than cattle. *Revue Scientifique et Technique*, 9(1), 131-150. https://doi.org/10.20506/rst.9.1.485.

Nol, P., Kato, C., Reeves, W.K., *et al.* (2010). Epizootic hemorrhagic disease outbreak in a captive facility housing white-tailed deer (Odocoileus virginianus), bison (Bison bison), elk (Cervus elaphus), cattle (Bos taurus), and goats (Capra hircus) in Colorado, U.S.A. *Journal of Zoo and Wildlife Medicine*, 41(3), 510-515. https://doi.org/10.1638/2009-0216.1.

Noronha, L.E., Cohnstaedt. L.W., Richt, J.A., & Wilson, W.C. (2021). Perspectives on the Changing Landscape of Epizootic Hemorrhagic Disease Virus Control. *Viruses*, *13*(11), 2268. https://doi.org/10.3390/v13112268.

Ohashi, S., Yoshida, K., Watanabe, Y., & Tsuda, T. (1999). Identification and PCR-restriction fragment length polymorphism analysis of a variant of the Ibaraki virus from naturally infected cattle and aborted fetuses in Japan. *Journal of Clinical Microbiology*, *37*(12), 3800-3803. https://doi.org/10.1128/JCM.37.12.3800-3803.1999.

Osburn, B.I. (1994). The impact of bluetongue virus on reproduction. *Comparative Immunology*,

Microbiology and Infectious Diseases, 17(3-4), 189-196. https://doi.org/10.1016/0147-9571(94)90042-6.

Qi, Y., Wang, F., Chang, J., *et al.* (2019). Identification and complete-genome phylogenetic analysis of an epizootic hemorrhagic disease virus serotype 7 strain isolated in China. *Archives of Virology*, 164(12), 3121-3126. https://doi.org/10.1007/s00705-019-04412-9.

Roug, A., Shannon, J., Hersey, K., Heaton, W., & van Wettere, A. (2022). Investigation into Causes of Antler Deformities in Mule Deer (Odocoileus hemionus) Bucks in Southern Utah, USA. *Journal of Wildlife Diseases*, 58(1), 222-227. https://doi.org/10.7589/JWD-D-21-00079.

Ruder, M.G., Johnson, D., Ostlund, E., *et al.* (2017). The First 10 Years (2006-15) of Epizootic Hemorrhagic Disease Virus Serotype 6 in the USA. *Journal of Wildlife Diseases*, 53(4), 901-905. https://doi.org/10.7589/2016-12-284.

Savini, G., Afonso, A., Mellor, P., *et al.* (2011). Epizootic heamorragic disease. *Research in Veterinary Science*, 91(1), 1-17. https://doi.org/10.1016/j.rvsc.2011.05.004.

Shope, R.E., Lester, G.M., & Robert, M. (1955). Deer Mortality-Epizootic Hemorrhagic Disease of Deer. *New Jersey Outdoors*, 6, 17-21.

Sick, F., Beer, M., Kampen, H., & Wernike, K. (2019). Culicoides Biting Midges-Underestimated Vectors for Arboviruses of Public Health and Veterinary Importance. *Viruses*, 11(4), 376. https://doi.org/10.3390/v11040376.

Şahin, S., Sabah, O.S., Elmas, L., Hacisalihoglu, U.P., & Yanik, S. (2023). Investigation of Toll-Like Receptor Family Expression in Glioblastoma: A Comparative Analysis of qPCR and Cell Culture. *Selcuk Medical Journal*, 39(2), 62-68.

Şevik, M. (2021). Genomic characterization of pestiviruses isolated from bovine, ovine and caprine foetuses in Turkey: A potentially new genotype of Pestivirus I species. *Transboundary and Emerging Diseases*, 68(2), 417-426. https://doi.org/10.1111/tbed.13691.

Temizel, E.M., Yesilbag. K., Batten. C., *et al*. (2009). Epizootic hemorrhagic disease in cattle, Western Turkey. *Emerging Infectious Diseases*, 15(2), 317-319. https://doi.org/10.3201/eid1502.080572.

Thabet, S., Sghaier, S., Hassine, B.T., *et al*. (2023). Characterization of Epizootic Hemorrhagic Disease Virus Serotype 8 in Naturally Infected Barbary Deer (Cervus elaphus barbarus) and Culicoides (Diptera: Ceratopogonidae) in Tunisia. *Viruses*, 15(7), 1567. https://doi.org/10.3390/v15071567.

Viarouge, C., Breard, E., Zientara, S., Vitour, D., & Sailleau, C. (2015). Duplex Real-Time RT-PCR Assays for the Detection and Typing of Epizootic Haemorrhagic Disease Virus. *PLoS One*, 10(7):e0132540. https://doi.org/10.1371/journal.pone.0132540.

WOAH, (2021). Epizootic Haemorrhagic Disease (Infection with Epizootic Hemorrhagic Disease Virus), Chapter 3.1.7. https://www.woah.org/fileadmin/Home/fr/Health_standards/tahm/3.01.07_EHD.pdf

Yadin, H., Brenner, J., Bumbrov, V., *et al.* (2008). Epizootic haemorrhagic disease virus type 7 infection in cattle in Israel. *The Veterinary Record*, 162(2), 53-56. https://doi.org/10.1136/vr.162.2.53.

Yang, H., Li, Z., Wang, J., *et al*., 2020. Novel Serotype of Epizootic Hemorrhagic Disease Virus, China. *Emerging Infectious Diseases*, 26(12), 3081-3083. https://doi.org/10.3201/eid2612.191301.

Yavru, S., Erol, N., Avcı, O., Esin, E., & Pasa, S. (2014). Isolation of epizootic hemorrhagic disease virus from sheep in western Turkey. *Revue de Medecine Veterinaire*, 165(1-2), 20-24.

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Evaluation of Sheep Colostrums According to Time after Lambing by Brix Refractometer Method and Color Scoring

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ABSTRACT

The aim of the study was to determine the effects of breed and post-lambing time on sheep colostrum quality. In this study, colostrum samples taken at different times after lambing from 4 different sheep breeds (Tuj, Awassi, Morkaraman, Akkaraman) raised in Turkey were compared using Brix refractometer and color scoring. The sheep included in the study were placed in different paddocks according to their breeds. In the first week after lambing, a total of 7 colostrum samples were collected from each ewe, every 12 hours for the first 3 days and on the 7th day (mature milk sample). According to the data obtained from the study, it was determined that colostrum quality was affected by breed and milking duration ($p<0.05$). Brix values of colostrum in the first and second milking were found to be higher in the Akkaraman breed than in the Morkaraman breed ($p<0.05$). Additionally, it was determined that the color score decreased over time after lambing and this decrease was statistically significant ($p<0.05$). In conclusion, since the quality of sheep colostrum varies depending on breed and time, the quality of the colostrum to be fed for lamb health and performance must be determined in advance.

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INTRODUCTION

Animal protein sources are of great importance for human health (Aktan and Uçar, 2022). Adequate and balanced nutrition is very important for optimal health (Alkan *et al.*, 2019). People need to consume animal protein daily to maintain a healthy and balanced diet. A significant portion of animal proteins consumed by humans are provided by ruminant animals, and sheep have an important place among them (Ölmez *et al.*, 2023). Sheep attract more attention because of shorter gestation period, higher twinning rate, and lesser slaughter age as well as comprise of better roughage utilization capacity than that of cattle. The survival of each lamb born in sheep farming also affects animal welfare, farm economy and the country's economy. (Cannas *et al.*, 2019).

The first secretion produced by the mammary glands immediately after birth is called colostrum (Pattinson *et al.*, 1995). Colostrum is a thick substance rich in nutrients that is secreted during the first 72 h after birth (Todaro *et al.*, 2023). Colostrum is very different from normal milk in terms of color and composition (Yılmaz and Kaşıkçı, 2013). The most important of these differences is the very high concentration of immunoglobulins in the colostrum, which boost the immune system of lambs. In addition, the level of casein, fat, protein, and vitamins (A, B_{12}, D and E) is higher in colostrum, but it is poorer in terms of lactose. Proteins, growth factors, immunoglobulins (e.g. IgG, IgM and IgE), hormones, cytokines, lactoferrin, interleukins, nucleosides and nucleotides which are important for the health of lambs are the main bioactive components of colostrum. The ratio of these components in the colostrum is higher in the first few hours after lambing (Todaro *et al.*, 2023).

Colostrum contributes to the development of the gastrointestinal tract by providing passive immunity for newborns. It also affects the endocrine and metabolic systems and serves as an energy source to protect newborn animals from hypothermia. (Rauprich *et al.*, 2000). It also has a laxative effect, helping to remove the meconium from the intestines (Pattinson *et al.*, 1995). It contains a trypsin inhibitor that prevents immunoglobulins, which are important for passive immunity, from being digested in the intestine (Yılmaz and Kaşıkçı, 2013; Kaçar and Batmaz, 2023). Transferrin and lactoferrin contained in colostrum prevent the growth of some pathogenic bacteria by binding iron and thus prevent the development of diarrhea. (Selk, 1998). Colostrum performs these functions with the support of cellular (leukocytes, lymphocytes, macrophages, and natural killer cells) and humoral (immunoglobulins, lactoferrin, lysozymes and complements) mechanisms (Yılmaz and Kaşıkçı, 2013). Sheep colostrum also contains insulin (INS), insulin-like growth factor I (IGF-1), prolactin (PRL), growth hormone (GH), thyroxine (T4) and triiodothyronine (T3) (Canapana and Baunarucker, 1995; Kaçar and Batmaz, 2023).

In particular, IgG found in the colostrum plays a key role in preventing passive transfer failure that may occur in newborns. In the early hours of life after birth, the intestines of newborn mammals allow large molecular structures of IgG to pass into the circulatory system. However, this feature disappears after few hours of birth (Tsioulpas *et al.*, 2007; Stelwagen *et al.*, 2009; Aytaç and Yazıcı; 2020). Since colostrum IgG concentration and intestinal IgG permeability reduce quickly in the first 24 hours after birth, it is vital for newborn animals to consume adequate quantity and quality of colostrum without delay (Moore *et al.*, 2005). Due to the structure of the placenta in ruminant animals, the transfer of immunoglobulin from mother to offspring is not carried out during pregnancy. Neonatal lambs and kids are born hypogammaglobulinemic and require colostrum containing immunoglobulin for immunological protection after birth (Dwyer *et al.*, 2016). It is the most sensitive and dynamic period of life for all newborns after the transition from the intrauterine environment to the extra uterine environment (Tokan and Geçkil, 2019; Altay, 2021). In order to ensure passive immunity, it is of great importance that newborn lambs consume sufficient amount and high-quality colostrum in the first hours

after birth. (Martins and Oliveira., 2020).

The quality of colostrum is very important for the profitableness of livestock enterprises as it affects the viability of newborns (Belkasmi *et al.*, 2022). Colostrum quality is influenced by many factors such as the animal's age, breed, pre-pregnancy nutrition level, length of drying period, dystocia, size and behavioral factors (Şireli, 2017). Accurate measurement of colostrum IgG level is essential to provide colostrum containing sufficient levels of immunoglobulin to newborn in animal farms for the continuation of a healthy generation (Baltrukova *et al.*, 2019). Additionally, for high-quality colostrum, it is recommended that the total number of bacteria and coliforms in the colostrum must be <100,000 cfu/ml and <10,000 cfu/ml respectively (Lago *et al.*, 2018). Colostrum IgG level can be determined by various methods. These methods; radial immunodiffusion (RID), hydrometer, refractometer, first milking weight, colostrum color, high performance liquid chromatography (HPLC), electrophoresis (Gapper *et al.*, 2007; Rivero *et al.*, 2012).

The purpose of this study was to compare color and Brix values with optical refractometer in colostrum samples collected at different times after birth from four different sheep breeds such as Awassi, Morkaraman, Akkaraman and Tuj, and to evaluate the effectiveness of Brix refractometers in sheep colostrum.

MATERIAL AND METHOD

Animal Materials and Experimental Groups

In this study, 28 sheeps obtained from a production farm (Awassi, Morkaraman and Tuj) affiliated with Ataturk University Food and Livestock Application and Research Center and a sheep farm (Akkaraman) in Erzurum, Türkiye, were used as experimental animals. The study was conducted in Erzurum province in northeastern Turkey during winter. Straw was used as litter material in the paddocks. Sheeps were selected by considering that they were healthy as well as their live weights, and body condition scores were also similar. The animals used in the study were included in the experiment considering that they were 2 or older. In addition, it was also ensured that all the animals used in the study were multiparous and gave birth to single offspring at each birth. Since the colostrum characteristics of the breeds were examined in the study, sheep of different breeds were placed in different paddocks. The study was conducted in 4 groups according to their breed, and with an equal number of sheep (7 per group) in each group. In the experimental trial, animals were fed with same diet (Table 1).

The animals in the experiment were given water, meadow grass and alfalfa grass ad libitum and were also fed 500 g of sheep milk feed. During the study, the feeding program implemented by the farm was applied to the animals, and no different feeding program was applied. Crude protein, crude ash and ether extract analyzes of the feeds used in the study were performed according to AOAC's Weende Analysis System (AOAC, 2005). NDF and ADF analysis of feeds was performed according to Van Soest and Robertson (1985) and Goering and Van Soest (1970). The current study was conducted with the guidelines and permission of Ataturk University Animal Experiments Local Ethics Committee, Erzurum, Turkey (E-36643897-000-2300020138).

Table 1. Nutrient composition (%)

Nutrient composition	%
Dry matter (%)	88
Crude protein (%)	14.4
Ether extract (%)	60
Acid detergent fiber (ADF) (%)	25.4
Neutral detergent fiber (NDF) (%)	38.9

Collection of colostrum and milk samples

All the colostrum samples were collected by the same person. After lambing the colostrum samples were collected into falcon tubes every 12 h for the first 3 days and on the 7th day (mature milk), a total of 7 samples were collected from each animal (Table 2). During colostrum samples collection, the colostrum (50 ml) that came after milking the udder several times was collected into sterile sample containers. The collected colostrum samples were brought to the laboratory in a cold chain and stored at -20^{o}C until further analysis (Abdel-Salam *et al.*, 2019). A backup copy (50 ml) of each colostrum sample was stored in another deep freezer (at -20^{o}C) until the analyzes were completed.

Table 2. Days and times to collect colostrum samples

Breed	1th hour (T0)	12th hour (T1)	24th hour (T2)	36th hour (T3)	48th hour (T4)	60th hour (T5)	Day 7 (mature milk sample-T6)
Tuj	x	x	x	x	x	x	x
Awassi	x	x	x	x	x	x	x
Morkaraman	x	x	x	x	x	x	x
Akkaraman	x	x	x	x	x	x	x

Brix refractometer analysis

Total protein concentration in the colostrum was determined at room temperature using a commercially available optical Brix refractometer (ATC Colostrum Refractometer S1310). Calibration and sample readings of the refractometer used during the study were performed in accordance with the manufacturer's instructions. (Santiago *et al.*, 2020). The brix percentage of the refractometer used varied between 0-30. All the colostrum samples were allowed to reach room temperature before refractometer analysis. The samples that reached room temperature were mixed homogeneously with a vortex and then measured with a refractometer and the results were recorded. The Brix refractometer analysis of all the colostrum samples was performed by a single person.

Color Analysis

The color scoring was performed as described by Prom *et al.*, (2022) using a scale of 1 to 4; 1 point was almost white (the color of milk) and 4 points were the color of orange juice.

Statistical analysis

Statistical analysis was performed using SPSS Statistics 20 (Statistical Package for the Social Sciences; SPSS Inc., Chicago/IL, USA). Pearson probability value (P value) was calculated using the One-Way ANOVA test to compare the data obtained from the study. A P value of less than 0.05 was considered statistically significant. The data obtained as a result of color scoring were subjected to

Kruskal-Wallis and Friedman tests.

RESULTS

In this study, the brix values and color scores of colostrum samples taken from different breeds of sheep at different times were examined. The brix values of colostrum are presented in Table 3, and the results obtained from color scoring are presented in Table 4.

Table 3. Change in colostrum Brix values (%) according to time after lambing

	T0	T1	T2	T3	T4	T5	T6
Tuj	24.8±1.02^{ab}	21.2±1.09^{ab}	16.9±1.05	15.9±0.46	13.9±0.61	14.4±0.52	13.8±0.29^{a}
Awassi	26.8±1.12^{ab}	22.7±1.78^{ab}	18.5±1.34	16.9±1.19	14.4±0.67	12.5±0.56	11.8±0.55^{b}
Morkaraman	23.0±2.01^{a}	17.9±1.0^{a}	15.8±0.57	15.2±0.65	14.7±0.62	13.6±0.62	12.5±0.43^{ab}
Akkaraman	28.3±0.9^{b}	24.3±1.02^{b}	19.0±1.27	18.0±0.63	16.1±0.41	15.4±0.34	12.3±0.34^{ab}

a-b: Means with different superscripts in the same column indicate significant differences ($p<0.05$).

Table 4. Change in colostrum color scores according to time after lambing

	T0	T1	T2	T3	T4	T5	T6
Tuj	3 (2-3)	2 (2-3)*	2 (1-2)*	1 (1-2)*	1 (1-1)^{a*}	1 (1-2)*	1 (1-1)^{a*}
Awassi	3 (3-4)	3 (2-3)*	3 (1-3)*	2 (1-4)*	2 (1-3)^{ab*}	2 (1-3)*	1 (1-2)^{ab*}
Morkaraman	3 (2-3)	3 (1-3)*	2 (1-3)*	2 (1-3)*	2 (1-2)^{b*}	2 (1-2)*	1 (1-1)^{a*}
Akkaraman	3 (2-4)	3 (2-4)*	2 (1-4)*	2 (1-2)*	2 (1-2)^{ab*}	2 (1-2)*	2 (1-2)^{b*}

a-b: Means with different superscripts in the same column indicate significant differences ($p<0.05$). * The same line shows the difference in color scoring according to time within the group regardless of breed ($p<0.05$). The values in parentheses indicate the smallest and largest values obtained in the measurements made on the relevant samples.

DISCUSSION

In this study, the changes in colostrum quality of different breed sheep over time were evaluated according to Brix values and color scoring, it was found that colostrum quality was affected by breed and milking time. Also, the brix values of colostrums at T0 and T1 milking were higher in the Akkaraman breed than in the Morkaraman breed. In addition, the comparison of the brix values of the samples collected that had turned into milk after one week of birth, showed that the samples taken from the Tuj breed had a higher brix value than those from the Awassi breed. As a result of the analysis of the data obtained, it was determined that there was a significant difference in color between the first colostrum samples taken after lambing (T0) and the samples taken on the 7th day (T6). In the comparisons made at the breed level, it was observed that the lowest quality was obtained in the Tuj breed sheep in the colostrum samples (T4) at the 5th milking (score 1) and that there was no significant difference between the other breeds. In the 7th sample (T6) taken at the end of the experiment, it was found that the colostrum with the highest score was from the Akkaraman breed, and the colostrum with the lowest score was from the Tuj and Morkaraman breeds.

In the literature review, it was observed that studies on determining the colostrum quality

focused mostly on dairy cows. In addition, the number of studies on the evaluation of colostrum quality in different sheep breeds using brix refractometer is limited (Castro *et al.*, 2018; Santiago *et al.*, 2020; Kessler *et al.*, 2021; Agenbag *et al.*, 2023). Therefore, this study was conducted to compare the colostrum qualities of four different domestic sheep breeds using Brix refractometers.

The high brix value in colostrum is directly related to the colostrum total protein content (IgG) (Kessler *et al.*, 2021). In a study conducted by Agenbag *et al.*, (2023) on South Australian Merino sheep, it was determined that the brix values were lower than those obtained from this study. In this study, brix % ranged from 21.6 to 44.7% at 0 hours postpartum, 15.1 to 45.3% at 4 hours, and 12.0 to 40.4% at 24 hours. However, the results obtained from this study were similar to those obtained by Kessler *et al.*, (2021). Kessler *et al.*, (2021) reported that in their study of increasing samples from their study comparing 10 different sheep and 10 different goat colostrums (Kessler *et al.*, 2019), the brix % ranged between 15.4-40.0. Kessler *et al.*, (2019) also stated that colostrum quality varies depending on breed. In a study conducted by Santiago *et al.*, (2020) on Santa Inés sheep, both colostrum quality and total serum protein levels of lambs were measured using a Brix refractometer. In this study, colostrum samples were collected at 6, 12, 24 and 48 hours after lambing and brix values were recorded. The results obtained were reported to be over 20% of the brix value in the first 24 hours. It was found that at the 48th hour after lambing, the brix value decreased to 15%. In addition, the ability to detect total serum protein levels with brix refractometers is another indication that brix refractometers are useful.

No studies have been conducted yet on the visual color scoring of colostrum samples in sheep. However, in a study conducted on Valle del Belice sheep (Todaro *et al.*, 2023), colostrum color analysis was performed with the help of "Chroma Meter". In this study, we tried to reveal the correlations between colostrum composition and color. In a study conducted to determine the effectiveness of β-carotene supplementation on colostrum in cattle during pregnancy, a color scoring system similar to that in our study was developed (Prom *et al.*, 2022). According to the study results, it was reported that as the color score increased, the brix value also increased. In our study, it was observed that the color score decreased as the brix value decreased over time. It was determined that the color score decreased with time after lambing and that this decrease was statistically significant. Additionally, it was observed that the colostrum samples taken from the Tuj breed were of lower quality than the T4 samples. In T6 samples, it was determined that the highest color score was in the Akkaraman breed.

CONCLUSION

In this study, it was determined that breed and the time elapsed after lambing were effective in improving the colostrum quality in sheep. It was thought that the difference between the obtained brix values and rank scores may be due to the colostrum IgG content. Brix refractometers used in the study is a useful, cheap and easy method that can be easily used by sheep breeders. Owing to the use of this method in sheep farming, lamb deaths due to consumption of poor quality colostrum can be prevented. Studies on colostrum quality in the literature have focused on dairy cows, and studies on sheep have been limited. Studies on the subject in sheep will make valuable contributions to the knowledge gap in the literature.

Ethical Approval

The current study was conducted with the guidelines and permission of Ataturk University Animal Experiments Local Ethics Committee, Erzurum, Turkey (E-36643897-000-2300020138).

Conflict of Interest

There is no Conflict of Interest. All authors contributed to the study conception and design. Material

preparation, data collection and analysis were performed by Soner UYSAL, Ayşe UYSAL, and Cihan ÖZ, Mükremin ÖLMEZ. The first draft of the manuscript was written by Ayşe UYSAL and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Additional information's

The summary of this study was presented at the 2023 Sheep Goat Health and Management Congress.

REFERENCES

Abdel-Salam Z.A., Abdel-Salam S.A.M., Abdel-Mageed I.I., & Harith M.A. (2019). Evaluation of proteins in sheep colostrum via laser-induced breakdown spectroscopy and multivariate analysis. *Journal of advanced research*, 15: 19-25.

Agenbag B., Swinbourne A.M., Petrovski K., & van Wettere W.H. (2023). Validation of a handheld refractometer to assess Merino ewe colostrum and transition milk quality. *Journal of Dairy Science*, 106(2): 1394-1402.

Aktan E. & Uçar A. (2022). Whey protein supplementation and its potential effects on health. *Journal of General Health Sciences*, 4(3): 318-329.

Alkan S.B., Solak R., Gürbüz E., *et al.* (2019). Eating behavior disorder and body perception in nutrition and dietetics department students: a cross-sectional study. *Necmettin Erbakan University Faculty of Health Sciences Journal*, 2(2): 38-44.

Altay G. (2021). Positioning practices for the baby in the neonatal intensive care unit. *Journal of General Health Sciences*, 3(2): 143-151.

AOAC: Official Methods of Analysis of AOAC International. 18th edn., Gaithersburg, USA, 2005.

Aytaç S.H., & Yazıcı S. (2020). The importance of breastfeeding and traditional practices in the postpartum period. *Necmettin Erbakan University Faculty of Health Sciences Journal*, 3(1): 21-26.

Baltrukova S., Zagorska J., & Eihvalde I. (2019). Evaluation of microbiological quality of colostrum. In 13th Baltic Conference on Food Science 'Food. Nutrition. 2-3 May. Jelgava, Letonya. 45–49.

Belkasmi F., Madani T., Mouffok C., & Semara L. (2022). Enzymatic quality of colostrum in Ouled Djellal ewes, Algeria. *Biological Rhythm Research*, 53(1): 1-9.

Canapana W., & Baunarucker C.I. (1995). Hormones and growth factors in bovine milk. Handbook of milk composition, 23, 476.California, USA. Academic Press.

Cannas A., Tedeschi L. O., Atzori A. S., & Lunesu M. F. (2019). How can nutrition models increase the production efficiency of sheep and goat operations?. *Animal Frontiers*, 9(2), 33-44.

Castro N., L.A. Gómez-González B. Earley and A Argüello. (2018). Use of clinic refractometer at farm as a tool to estimate the IgG content in goat colostrum. *Journal of Applied Animal Research*, 46:1505–1508.

Dwyer C.M., Conington J., Corbiere F., *et al.* (2016). Invited review: Improving neonatal survival in small ruminants: Science into practice. *Animal*, 10(3): 449-459.

Gapper L.W., Copestake D.E.J., Otter D.E., ve Indyk H.E. (2007). Analysis of bovine immunoglobulin G in milk, colostrum and dietary supplements: A review. *Analytical and Bioanalytical Chemistry*, 389(1): 93109.

Goering H.K., & Van Soest P.J. (1970). Forage fiber analyses (apparatus, reagents, procedures, and some applications) (No. 379). US Agricultural Research Service.

Kaçar Y., & Batmaz H. (2023). Evaluation of the differences in proteomics of high-quality bovine colostrum and low-quality bovine colostrum. *Veterinary Medicine and Science*, 9(6): 2852-2861.

Kessler E.C., Bruckmaier R.M., & Gross J.J. (2019). Immunoglobulin G content and colostrum composition of different goat and sheep breeds in Switzerland and Germany. *Journal of Dairy Science*, 102(6): 5542-5549.

Kessler E.C., Bruckmaier R.M., & Gross J.J. (2021). Comparative estimation of colostrum quality by Brix refractometry in bovine, caprine, and ovine colostrum. *Journal of Dairy Science*, 104(2): 2438-2444.

Lago A., Socha M., Geiger A., *et al.* (2018). Efficacy of colostrum replacer versus maternal colostrum on immunological status, health, and growth of preweaned dairy calves. *Journal of Dairy Science*, 101(2): 1344–1354.

Martins L., & Oliveira L. (2020). Colostrum as the speed up key for ruminant newborn: what do we know and should further characterize. *Journal of Dairy, Veterinary & Animal Research*, 2020; 9(3): 95–98.

McGrath B.A., Fox P.F., McSweeney P.L., & Kelly A.L. (2016). Composition and properties of bovine colostrum: a review. *Dairy Science & Technology*, 96: 133-158.

Moore M., Tyler J.W., Chigerwe M., Dawes M.E., & Middleton J.R. (2005). Effect of delayed colostrum collection on colostral IgG concentration in dairy cows. *Journal of the American Veterinary Medical Association*, 226(8): 1375-1377.

Ölmez M., Riaz R., Karadağoğlu Ö., *et al.* (2023). Effect of SOD-Rich Melon Supplement on Performance, Serum Biochemical, Antioxidant and Meat Quality Characteristics of Tuj Lambs. *Agriculture*, 13(3), 625.

Page P., Sherwin G., Sampson R., Phillips K., & Lovatt F. (2022). Ewe colostrum quality on commercial Welsh sheep farms. *Livestock*, 27(1): 40-46.

Pattinson S.E., Davies D.A.R., & Winter A.C. (1995). Changes in the secretion rate and production of colostrum by ewes over the first 24 h post partum. *Animal Science*, 61(1): 63-68.

Prom C.M., Engstrom M.A., & Drackley J.K. (2022). Effects of prepartum supplementation of β-carotene on colostrum and calves. *Journal of Dairy Science*, 105(11): 8839-8849.

Rauprich A.B.E., Hammon H.M., & Blum J.W. (2000). Influence of feeding different amounts of first colostrum on metabolic, endocrine, and health status and on growth performance in neonatal calves. *Journal of Animal Science*, 78(4), 896-908.

Rivero M.J., Valderrama X., Haines D., Alomar D. (2012). Prediction of immunoglobulin G content in bovine colostrum by near-infrared spectroscopy. *Journal of Dairy Science*, 95(3): 1410–1418.

Santiago M.R., Fagundes G.B., do Nascimento D.M., *et al.* (2020). Use of digital Brix refractometer to estimate total protein levels in Santa Inês ewes' colostrum and lambs' blood serum. *Small Ruminant Research*, 182: 78-80.

Selk G.E. (1998). Management factors that affect the development of passive immunity in the newborn calf. In: Beef Cattle Handbook-2240. Extension Beef Cattle Resource Committee. 1–7. Oklahoma, USA. Oklahoma State University.

Stelwagen K., Carpenter E., Haigh B., Hodgkinson A., & Wheeler T.T. (2009). Immune components of bovine colostrum and milk. *Journal of Animal Science*, 87(suppl_13): 3-9.

Şireli H.D. (2017). The Importance of Colostrum in the Growth of Lambs and Kids. *Dicle University Faculty of Veterinary Medicine Journal*, 10(2): 168–172.

Todaro M., Maniaci G., Gannuscio R., Pampinella D., & Scatassa M. L. (2023). Chemometric Approaches to Analyse the Composition of a Ewe's Colostrum. *Animals*, 13(6): 983.

Tokan F., & Geçkil E. (2019). The Concept of Collective Caregiving within the Context of Individualized Developmental Care in Premature Babies. *Journal of General Health Sciences*, 1(1): 64-77.

Tsioulpas A., Grandison A.S., & Lewis M.J. (2007). Changes in physical properties of bovine milk from the colostrum period to early lactation. *Journal of Dairy Science*, 90(11): 5012-5017.

Van Soest P.J., & Robertson J.B. (1985). Analysis of forages and fibrous foods. Cornell University.

Yılmaz Ö., & Kaşıkçı G. (2013). Factors affecting colostrum quality of ewes and immunostimulation. *Turkish Journal of Veterinary & Animal Sciences*, 37(4): 390-394.

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Effects of Gender on Hematologic Parameters in Kangal Shepherd Dogs

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ABSTRACT

Kangal Shepherd Dogs are highly valuable working dogs unique to Turkey, known for their robustness in withstanding harsh climatic conditions and possessing advanced herd protection and management abilities due to their genetic characteristics. Haematological values serve as crucial indicators of the physiological and physiopathological parameters in animals. A complete blood cell count (CBC) emerges as a powerful diagnostic tool when appropriately assessed. Given the establishment of pure breeds, it is essential to acknowledge that breed-specific hematologic values may exhibit variations influenced by factors such as gender, lifestyle, age, and geographical location. Therefore, understanding the physiological distinctions between genders becomes paramount. This study aims to assess the impact of gender on the complete blood count in Kangal Shepherd Dogs. Upon comparing hematologic parameters between female and male Kangal Shepherd Dogs, a statistically significant difference was observed in the mean platelet volume (MPV) value, which was higher in females than males ($p < 0.05$). While the mean red blood cell (RBC), haemoglobin (HGB), and haematocrit (HCT) values were higher in males, no statistically significant results were found ($p > 0.05$).

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INTRODUCTION

It is known that the domestication of gray wolves produced the ancestors of the first dogs capable of herd protection (Galibert, 2011). Kangal shepherd dog breed is intensively preferred for both herd protection and guard duties in Turkey as in many countries around the world (Kockaya, 2019). Kangal shepherd dogs have a genotype and specific phenotypic characteristics unique to Turkey (Erdoğan *et al*, 2013). It is reported to be the only dog breed that is durable enough to withstand harsh climatic conditions in nature, has highly developed herd protection and management abilities, and can even neutralize wolves attacking the herd (Özbeyaz, 1994). Kangal shepherd dogs have a characteristic appearance with lioness-like body lines, a black mask around the mouth and eyes, and a tail curled up and forward (Yılmaz, 2007).

Hematological values are an important source of information about physiological and physiopathological parameters of animals (Barger, 2003). Complete blood cell count (CBC) is a powerful diagnostic tool (Dixon, 1997). When CBC is fully and appropriately evaluated, it allows a diagnosis to be made or a differential diagnosis list to be created (DeNicola, 2011). Reference values for animal species are important in this assessment (Etim *et al*, 2014). It has been stated in different studies that characteristics such as lifestyle, gender, age and climate may cause differences in hematological parameters (Connolly *et al*, 2020; Olayemi & Ighagbon, 2011). It is known that Kangal shepherd dogs are used as working dogs for herd protection and are raised as breeding dogs in various dog farms (Özbeyaz, 1994).

It is thought that increased intraspecies homogeneity, the presence of specific behavioral patterns and similarity of lifestyles may lead to increased interspecies genetic differences and variation in hematological reference values within species (Alilovic *et al*, 2022). It is thought that dogs used in herd management and guarding, such as Kangal shepherd dogs, may have hematologic differences compared to small breed dogs and domestic animals due to different lifestyles (Gavazza *et al*, 2012; Lee *et al*, 2020). Breed appears to be an important factor for the appropriateness of population-specific reference ranges and should be considered when performing health examinations including hematologic and biochemical parameters. It is thought that gender factor may also be an important variable in intra-breed evaluations.

This study was carried out to evaluate the effects of gender on hematologic parameters in kangals bred in farms and to establish reference values for complete blood count evaluation of kangal shepherd dogs.

MATERIALS AND METHODS

Animals

The scope of the study consisted of a total of 38 dogs, 20 healthy and adult dogs older than 6 months and younger than 6 years of age, 20 mother dogs and 17 breeding dogs, which were bred in the Anatolian Shepherd Dogs & Kangal Farm in Kangal farm in Eldivan District of Çankırı province. After the anamnesis of the dogs were taken, physical examinations were completed and those who were determined to be healthy were included in the study.

Blood samples

In order to eliminate the changes that may be caused by stress in the dogs included in the study,

the dogs were taken to the areas where they normally lived with people they knew and trusted. The dogs were allowed to get used to and trust them to researchers before blood sampling and spent 20 minutes in the cage. During this period, anamnesis was obtained from the animal caregiver and a physical examination was performed. After the physical examination was completed, blood was collected aseptically from *V. cephalica* into tubes containing K2 EDTA as anticoagulant and blood was counted in a fully automatic blood counting device (Abacus Junior Vet5). With this blood count, 22 different values measured with the device at one time were obtained. These are respectively; White blood cells (WBC), Lymphocytes (LYM), Monocytes (MON), Neutrophils (Neu), Eosinophils (EOS), Lymphocyte percentage (LYM%), Monocyte percentage (MO%), Neutrophils percentage (NE%), Eosinophils percentage (EO%), Basophils percentage (BA%), Red Blood Cell (RBC), Hemoglobin (HGB), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red cell distribution width (RDW), Platelets (PLT), Platelet-crit (PCT), Mean platelet volume (MPV), Platelet distribution width (PDW).

Statistics

Descriptive statistics of the data were calculated and Arithmetic Mean, Standard Error, Standard Deviation, Median, Minimum and Maximum values were calculated and presented in a table. Parametric assumptions were tested by Shapiro-Wilk Test and Levene's Test. Since MON, NEU, MO%, RDWc, PLT, PCT variables did not meet the parametric assumptions, Mann-Whitney U test was performed for differences between gender groups. Since the other variables met the parametric assumptions, Student's T test was performed for the differences between gender groups. The analysis was performed at 0.05 significance level. IBM SPSS 23.0 statistical package program was used for all statistical analyses.

RESULTS

This study focused on the effect of gender on complete blood count values in healthy Kangal shepherd dogs. The hematologic values obtained from the dogs included in the study are given in Table 1. When hematologic parameters were compared between female and male Kangal shepherd dogs, it was observed that mean platelet volume (MPV) value was statistically significantly higher in females than males ($p<0.05$). The mean RBC, HBG and HCT values were higher in males, but no statistically significant result was determined ($p>0.05$). The data we obtained were compared with the cbc reference values of dogs. In the comparison, it was determined that RBC, HBG, HCT, PDW parameters of male dogs and WBC, EOS, HGB, PCT and PDW parameters of female dogs were outside the reference range (Table 1).

DISCUSSION

CBC is one of the most basic hematologic diagnostic methods used by clinicians (Dixon, 1997). When evaluated appropriately, it provides important information about the health status of the animal, definitive diagnosis of diseases and preparation of a differential diagnosis list (DeNicola, 2011). It is important to know the specific reference values for species in the evaluation of complete blood count (Etim *et al*, 2014). Today, the breeding of pure breeds within species and the awareness of breeders about the importance of preserving the genotypes of breeds have led to an increase in genetically determined differences between breeds. It is thought to have breed-specific hematological values due to the creation of homogeneous populations in terms of phenotype, genotype and behavioral characteristics

Table 1. The effect of gender on total blood count results in Kangal shepherd dogs.

		GENDER												P
		Male						Female						
Parameters	**Units**	x̄	SE	S	Mean	Minimum	Maximum	x̄	SE	S	Mean	Minimum	Maximum	
WBC	**x 10^9/L**	15,89	0,81	3,34	16,67	10,85	21,32	17,89	1,12	5,00	17,09	10,83	30,24	0,169
LYM	**x 10^9/L**	3,37	0,52	2,16	2,30	0,65	7,09	3,21	0,35	1,55	3,24	1,02	5,59	0,799
***MON**	**x 10^9/L**	0,67	0,09	0,36	0,54	0,13	1,61	0,89	0,13	0,57	0,78	0,26	2,40	0,175
***NEU**	**x 10^9/L**	11,27	0,66	2,71	10,98	6,50	16,69	12,81	0,87	3,91	12,89	6,60	23,76	0,180
EOS	**x 10^9/L**	0,48	0,06	0,23	0,52	0,09	1,01	0,66	0,10	0,43	0,53	0,19	1,72	0,124
BAS	**x 10^9/L**	0,10	0,02	0,08	0,08	0,01	0,29	0,14	0,02	0,10	0,12	0,03	0,45	0,145
LY%	**%**	20,6	2,9	12,0	17,2	5,3	42,6	18,4	1,8	8,3	18,6	5,0	35,1	0,531
***MO%**	**%**	4,3	0,5	2,2	4,0	0,8	9,8	5,0	0,5	2,3	5,1	1,5	9,2	0,329
NE%	**%**	71,5	2,8	11,7	71,1	48,6	87,7	71,7	2,3	10,3	71,2	53,6	84,8	0,965
EO%	**%**	3,1	0,3	1,3	3,2	0,6	5,2	4,1	0,6	2,8	3,3	1,1	11,8	0,160
BA%	**%**	0,6	0,1	0,5	0,5	0,1	1,5	0,8	0,1	0,4	0,8	0,2	1,5	0,198
RBC	**x 10^{12}/L**	8,84	0,25	1,04	8,90	6,77	10,49	8,39	0,31	1,37	8,35	6,21	10,42	0,271
HGB	**g/dl**	20,1	0,7	2,7	20,2	15,1	23,9	19,3	0,8	3,6	18,8	14,3	24,5	0,454
HCT	**%**	55,30	2,31	9,50	58,41	28,43	65,68	54,54	2,05	9,15	55,19	41,67	67,95	0,806
MCV	**fL**	64,5	0,7	2,6	64,5	59,0	70,0	65,0	0,6	2,7	65,0	60,0	71,0	0,618
MCH	**pg**	22,70	0,15	0,60	22,75	21,70	24,00	23,06	0,23	1,02	22,80	21,00	25,70	0,221
MCHC	**g/dl**	35,0	0,5	2,0	34,8	30,6	38,6	35,3	0,3	1,2	35,0	33,6	38,2	0,552
***RDW**	**%**	17,1	0,2	1,0	17,0	15,9	19,4	16,8	0,1	0,6	16,5	16,0	18,1	0,433
***PLT**	**x 10^9/L**	220	35	140	185	31	534	246	23	102	247	37	445	0,152
***PCT**	**%**	0,25	0,04	0,17	0,20	0,03	0,65	1,46	1,19	5,31	0,27	0,03	24,00	0,161
MPV	**fL**	10,3	0,2	0,8	10,3	9,4	11,9	11,0	0,2	1,1	11,0	8,9	13,1	0,023
PDW	**%**	39,8	0,8	3,1	40,5	30,2	43,8	41,4	0,3	1,3	41,6	39,2	44,0	0,054

x̄: Arithmetic mean, SE: Standard Error, S: Standard Deviation, *: Variables for which Mann-Whitney U test was performed.

within pure breeds and the production of strong breeders in this direction (Alilovic *et al*, 2022). It should also be kept in mind that breeds can be affected by various variables such as age, gender, lifestyle and geographic location (Gavazza *et al*, 2012; Lee *et al*, 2020). It is important to consider the physiological differences between genders. In this study, we evaluated the effects of gender on complete blood count in kangal shepherd dogs.

Mean platelet volume (MPV), which represents the average platelet size, is a routinely studied parameter (Bommer *et al*, 2008). Several studies have been conducted on the diagnostic value of MPV in dogs. It was reported that MPV increased significantly in experimentally induced endotoxemia in dogs (Yilmaz *et al*, 2008). It was also reported that MPV increased in dogs with babesiosis (Žvorc *et al*, 2010). Schneider and Mischke (2016) and Nikolic et al. (2022) reported that gender affected the MPV parameter statistically significantly ($p<0.05$) in healthy dogs in accordance with our study. However, while higher MPV values were determined in male dogs compared to female dogs in the reported studies, a statistically significant higher value was determined in females in our study. However, in contrast to these studies, Bommer et al (2008) reported that gender had no significant effect on MPV in a study with 80 control and 159 thrombocytopenic dogs. In our study, although MPV was statistically significant, no significant results were observed in PLT, PCT and PDW parameters (Table 1). It is thought that interpreting only MPV for platelet size and distribution may be misleading by the authors.

Kockaya et al. (2021) investigated the effects of gender and age on hematologic parameters in Kangal shepherd dogs and reported that there was no significant relationship between sex, age and age-sex relationship in hematologic parameters, which is partially consistent with our study. In accordance to our study, it was reported that MPV in adult Kangal shepherd dogs was higher in females, but gender was not statistically significant (Kockaya *et al*, 2021). It is thought that the increase in the number of samples in our study was effective in the emergence of a statistically significant expression.

According to the similar study conducted by Kockaya et al. (2021) in Kangal shepherd dogs, when the CBC parameters were compared on the basis of gender with our study, it was determined that RBC, HGB, MCHC, RDW, MPV and PDW parameters were higher in both male and female Kangal dogs and PCT parameter was higher only in female Kangal dogs. It is thought that this situation may be related to complete blood count device.

CONCLUSION

The Kangal shepherd dog is a working dog native to Turkey with a very high herding ability. Hematologic data may vary in various situations. This study investigated the effects of gender on CBC. The results showed that gender had no statistically significant effect, except for the MPV parameter. More studies are needed to better understand the physiology and physiopathology of Kangal shepherd dogs. Further comprehensive and different studies may help veterinarians to avoid misinterpretation of laboratory results in the diagnostic process, prognosis and therapeutic monitoring.

Ethical Approval

The Kırıkkale University Animal Experiments Local Ethics Committee (2023/04-15) approved and deemed the study's animal experimentation protocols compliant.

Conflict of Interest

The authors declared that there is no conflict of interest. Material preparation and data collection were carried out by Halime Kara and Yasin Şenel. Statistical analysis was carried out by Ali Alparslan Sayım.

The first draft of the manuscript was written by Mustafa Güven, and all authors commented on previous versions of the manuscript. All authors read and approved the final version of the manuscript.

REFERENCES

Alilovic, I., Rukavina, D., Ajanovic, A., Crnkic, C., Ohran, H., & Zahirovic, A. (2022). Breed, age, and sex-related variations in hematological and some biochemicalparameters in the Tornjak dog. *Turkish Journal of Veterinary & Animal Sciences*, 46(2), 192-200.

Barger, A. M. (2003). The complete blood cell count: a powerful diagnostic tool. Veterinary Clinics: *Small Animal Practice*, 33(6), 1207-1222.

Bommer, N. X., Shaw, D. J., Milne, E. M., & Ridyard, A. E. (2008). Platelet distribution width and mean platelet volume in the interpretation of thrombocytopenia in dogs. *Journal of small animal practice*, *49*(10), 518-524.

Connolly, S. L., Nelson, S., Jones, T., Kahn, J., & Constable, P. D. (2020). The effect of age and sex on selected hematologic and serum biochemical analytes in 4,804 elite endurance-trained sled dogs participating in the Iditarod Trail Sled Dog Race pre-race examination program. *Plos one*, *15*(8), e0237706.

DeNicola, D. B. (2011). Advances in hematology analyzers. *Topics in companion animal medicine*, 26(2), 52-61.

Dixon, L. R. (1997). The complete blood count: physiologic basis and clinical usage. *The Journal of perinatal & neonatal nursing*, 11(3), 1-18.

Erdoğan, M., Tepeli, C., Brenig, B., et al. (2013). Genetic variability among native dog breeds in Turkey. *Turkish Journal of Biology*, 37(2), 176-183.

Etim, N. N., Williams, M. E., Akpabio, U., & Offiong, E. E. (2014). Haematological parameters and factors affecting their values. *Agricultural science*, 2(1), 37-47.

Galibert, F., Quignon, P., Hitte, C., & André, C. (2011). Toward understanding dog evolutionary and domestication history. *Comptes rendus biologies*, 334(3), 190-196.

Gavazza, A., Rispoli, D., Bernabò, N., & Lubas, G. (2012). Retrospective and observational investigation of canine microcytosis in relationship to sex, breed, diseases, and other complete blood count parameters. *Comparative Clinical Pathology*, 21, 545-553.

Kockaya, M., Ekici, M., Genç, E., Çelikgün, S., & Başekici, H. (2021). The Effect of Age and Gender on Some Hematological Parameters and Erythrocyte Osmotic Fragility in Kangal Shepherd Dogs. *Atatürk Üniversitesi Veteriner Bilimleri Dergisi*, *16*(3), 298-305.

Lee, S. H., Kim, J. W., Lee, B. C., & Oh, H. J. (2020). Age-specific variations in hematological and biochemical parameters in middle-and large-sized of dogs. *Journal of veterinary science*, 21(1).

Nikolic, S., Belic, B., Cincovic, M., Novakov, N., Plavsa, N., & Savic, S. (2022). The effects of biological and health characteristics of dogs on intraindividual variabilityof blood parameters. *Turkish Journal of Veterinary & Animal Sciences*, 46(3), 385-395.

Olayemi, F. O., & Ighagbon, F. O. (2011). Haematology of the German Shepherd dog in a humid tropical environment. *Comparative clinical pathology*, *20*, 61-64.

Özbeyaz, C. (1994). Kangal Köpeklerinde Bazı Morfolojik Özellikler. *Lalahan Hayvancılık Araştırma Enstitüsü Dergisi*, *34*(1-2), 38-46.

Schneider, L., & Mischke, R. (2016). Platelet variables in healthy dogs: reference intervals and influence of age, breed and sex. *Comparative Clinical Pathology*, *25*, 1097-1106.

Yılmaz, O. (2007). Turkish kangal (Karabash) shepherd dog. *Impress Printing Comp. Ankara (Turkey)*.

Yılmaz, Z., Eralp, O., & Ilcol, Y. O. (2008). Evaluation of platelet count and its association with plateletcrit, mean platelet volume, and platelet size distribution width in a canine model of endotoxemia. *Veterinary clinical pathology*, *37*(2), 159-163.

Žvorc, Z., Barić Rafaj, R., Kuleš, J., & Mrljak, V. (2010). Erythrocyte and platelet indices in babesiosis of dogs. *Veterinarski arhiv*, 80(2), 259-267.

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The Use of Statistics in Veterinary Sciences and The Test Methods Used

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ABSTRACT

In this study, the statistical methods mostly used in veterinary sciences were examined. Currently, a statistical audit unit is being established in all sciences and scientific journals in terms of the reliability of the study. This unit determines the reliability of the study as a result of this control by checking whether the correct method is used. Statistics starts at the design stage of the experiment and reaches the right results with the right methods. If the statistical plan is not made at the beginning of the trial, the desired results cannot be achieved. For these reasons, it would be useful to get support from a statistician in order to achieve the desired results in studies of veterinary sciences.

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INTRODUCTION

As in all experimental research sciences, statistical methods are used to analyse study results in the field of veterinary science. Statistics in general terms; production, consumption, population, health, education, agriculture, traffic, etc. in subjects, it is based on collections of data showing facts and the evaluation of these data. It is interpreted by determining whether the study results cause a significant change or how effective it is as a result of a statistical evaluation. However, the decisions and comments made in scientific studies where results are obtained using any statistical methods are shaped by the results obtained as a result of the study. Therefore, whether a decision is correct or not depends not only on the use of statistics, but also on whether the statistical method used was chosen correctly and how accurately the analysis was performed. For this reason, it is extremely important to evaluate scientific articles in terms of statistical use (Bayir and Tekin, 2022).

Veterinary science is mostly based on experimental studies. Statistical analysis is definitely required to determine the result of the experiment performed. Although there are no comparative statistics in most other sciences, it is possible to explain the results of the study using descriptive statistical methods. On the basis of these explanations which depends on the results of the study, it is accepted by the researchers. In veterinary science, however, research is supported by both descriptive and comparative analyses.

Statistics provide objectivity. When evaluating living things with the same characteristics in two different environments, statistics reveal the difference of the environment on the living thing. Conversely, in the case of different living things in the same environment or experiment, it reveals the difference of a living being. If it is the same living being and in the same environment, differences that are outside the researcher's control arise. It is necessary to repeat the study to minimise these errors. Thanks to these repetitions and safe analyses, statistical programs also provide us. In order to obtain unbiased estimates of treatment averages and trial error, the chance of one treatment falling into any trial unit must be equal to the chance of falling into another trial unit. In a well-designed experiment, it is necessary to look for features in which there is no simple, systematic error, the degree of certainty is high, and the range of validity of the results is wide (Efe *et al*., 2000).

The accuracy of statistical estimates depends on the accuracy of the figures analyzed in one place and the experimental design chosen, while the accuracy of the figures depends on the honesty of the researcher and the sensitivity he shows in his work. In this case, a researcher should definitely consult a statistician or a person who knows the subject if he does not have enough information to set up an experiment suitable for his purpose before setting up the experiment and obtaining the figures (Kayaalp and Şahinler, 1996). Otherwise, consultation after obtaining the figures often does not provide any benefit. This is because no statistical method can literally eliminate the errors that are made İn setting up the experiment. As a result, the analyses performed are scientifically incorrect or inaccurate, and the person who is consulted later becomes a partner in the scientific error made. Therefore, a researcher should consider the following issues before starting his scientific research (Bek and Efe, 1988).

1. Accurately describing the problem that is the subject of research,
2. The purpose of the study should be clearly defined,
3. To organize the problem that is the subject of the study within the framework of criticism by discussing with experts,
4. Determination of the treatments to be used in the solution of the problem that is the subject of the study,
5. The best representation of the trial material for the population,

6. Choosing a trial plan that is suitable for the purpose,
7. Determination of the number of repetitions of the experimental units to be observed,
8. The place, time, manner in which the measurements were made, etc. deciding what to do,
9. Performing statistical analysis and determining the way the results are summarized,
10. Establishment of the experiment in the light of the above mentioned,
11. Analysis after obtaining the data and interpretation of the results,
12. The result of the research should be reported clearly and clearly. Providing the necessary conditions at each of these stages will increase the scientific value of the study.

Group Comparison Tests

It is used in simple experiments where two different treatments are applied, or two different groups are compared. The hypotheses put forward in the experiment are tested using T or Z distributions depending on the number of data. A hypothesis is defined as claims or statements that are put forward about one or more populations from a statistical point of view and are possible to be true or false. When making a decision about a population, because of the population size, we have to conduct our test based on the limited amount of information provided by a sample. In order for a scientist to objective, he or she must test the claim or hypothesis put forward on the basis of evidence. For this purpose, while the "comparison of the average of two groups" method is used when comparing two different treatments, if there is a relationship between the trial units in the groups, then the "Paired Comparison Method" should be used (Akar and Şahinler, 1993).

The Method of Analysis of Variance

Analysis of variance is a statistical approach that uses whether there is a difference between the means of two different groups by using variance. Many researchers in veterinary medicine use this method. Although there are different methods used to compare the two groups (Z or T test), the most common one is the F test, i.e. Analysis of Variance (ANOVA, Analysis of Variance) (Ervural, 2020). If the number of groups to be compared is more than two, or if there are interactions between treatments, the method of variance analysis should be used.

There are some issues that should be taken into account when applying the variance analysis method. In short, they are as follows.

- Before performing a variance analysis test, variance homogeneity tests such as Fmax, Cochran, Bartlett belonging to the groups should be performed.

- If there is no homogeneity between the variances of the groups as a result of the test performed, variance analysis should be applied after converting the data with the relevant transformations (such as Logarithmic, Square Dec, Angle transformation). If the validity of the assumptions cannot be ensured by transformations, non-parametric statistical techniques should be used.

- At the beginning of the experiment, an appropriate mathematical model should be selected depending on the purpose and conditions. Otherwise, the whole experiment will be wrong.

If the effects of variables that can only be measured are investigated in the analysis, multiple regression analysis is performed, and covariance analysis is performed if the effects of independent variables that can be measured and unmeasured are investigated. In addition, the effect of variables that can also be measured by multiple regression analysis and those that cannot be measured on the

dependent variable is included in the analysis by introducing the unmeasured variable as a “dummy” variable (Büyüköztürk, 1997; Nie, 1976). In single-factor variance analysis, there is an independent variable and a dependent variable. In the two-factor variance analysis, there are two independent variables and one dependent variable. The main purpose here is to investigate the joint effect of the independent variables on dependent variables. In addition, the means of each independent variable and its corresponding group are compared according to the dependent variable, and it is investigated whether the difference between the means is significant according to their meaning Deciency.

By using the variance analysis tables in veterinary medicine, the variance elements used in the estimation of genetic parameters in animal breeding can also be estimated. In addition to using variance analysis tables to estimate variance elements, "Constrained Maximum Likelihood Method (REML)", "Maximum Likelihood Method (ML)", "Quadratic Deviation-Free Prediction Method with Minimum Variance (MIVQUE)" are also used (Kayaalp and Bek, 1994).

Nonparametric Statistics

If the assumptions of the variance analysis are not met, or if the variances between the groups are heterogeneous after the transformation is applied to the data, then the statistics to be applied are non-parametric statistics (Gamgam and Altunkaynak, 2008). If the variances between the groups are heterogeneous again, then the statistics to be applied are non-parametric statistics.

Non-parametric statistics can be used for the specified situations in veterinary medicine, as well as for various survey studies. For example, if it is desired to investigate whether there is a Deciency between calf deaths in livestock and the drugs used on the mother, the Chi Square test statistic or the Median test can be used. In addition, there are many non-parametric statistics used in veterinary medicine (such as the Kruskal Wallis Test, Mann Whitney U Test, Fridman Test, Spermann's Rank Correlation Test). It can also make use of these statistics in the analysis of the results evaluated by coding.

Kruskal Wallis One-Way Variance Analysis is the most widely used test for the null hypothesis, which claims that “more than two independent samples were drawn from the same main masses” and is a good alternative to one-way variance analysis. The alternative hypothesis is that “The median of at least one main mass is different from that of the other main masses” (Ruxton-Beauchamp 2008, Karagöz *et al*., 2009).

Mann-Whitney U Test; This test is a non-parametric alternative to the t-test applied for independent samples. instead of comparing the averages of two groups as in the t-test, the Mann-Whitney U test compares the medians of the groups. It converts the values of continuous variables into sequential within two groups. Thus, it evaluates whether the ranking between the two groups is different or not space. Since the values are transformed into a sequential form, the actual distributions of the values are not important (Kalaycı *et al.* 2006). The data must be at least on the ordinal scale. The null hypothesis takes the form of “the samples were taken from the same main audience or the main audiences from which the samples were taken are not different from each other”.

The Friedman test is a nonparametric alternative to two-way analysis of variance. Data obtained by sequential, score or interval scale for k transactions from a group is used to test the effects of transactions. Ranking points are used instead of actual observations. The null hypothesis is “transactions have no effect”, while the alternative hypothesis is “transactions have different effects” (Özdamar, 2004; Keller-Warrack, 2003).

The Spearman rank correlation coefficient test determines the relationship and the degree of the relationship. This test is a nonparametric alternative of pearson correlation coefficient. The data should be obtained independently, randomly and with at least an ordinal scale. The null hypothesis is "Events are independent of each other, they do not affect each other, there is no relationship between them", while the alternative hypothesis is "Events are not independent of each other space Decently, they affect each other, there is a relationship between them".

Multiple Comparison Tests

Multiple comparison tests are complementary techniques for analysis of variance. In this case, the validity of these tests depends on the validity and accuracy of the analysis of variance, in which the population means, and error variances used as test parameters are obtained. If a statistical difference has been found between the groups SpaceDecently as a result of the F test carried out together with the analysis of variance, one of the tests such as Lsd, Duncan, Snk, Tukey, Dunnet, Scheffe is used to reveal the difference between these groups.

The Least Significant Difference Method (LSD)

The Least Significant Difference Method (LSD), proposed by Fisher (1935), is used to make all possible binary comparisons between group means when the control hypothesis is rejected as a result of analysis variance. The Least Significant Difference Method (LSD) is used to make all possible binary comparisons between group averages. It is the most widely used method among multiple comparison methods, the easiest to use, but the least reliable. It checks the error per comparison (Düzgüneş *et al.*, 1987; Milliken and Johnson, 1992; Soysal, 2000; Kesici and Kocabaş, 2007; Montgomery 2008; Şenoğlu and Acıtaş 2010).

The Duncan Method

Another method proposed to make all possible binary comparisons between group means is the multiple range test (multiple range test), proposed by Duncan (1955). The Duncan method takes into account the position of the means in the order according to their sizes when comparing the group means (Düzgüneş *et al*., 1987; Milliken and Johnson, 1992; Soysal, 2000; Kesici and Kocabaş 2007; Montgomery, 2008; Şenoğlu and Acıtaş 2010).

Student Newman Keuls Method (SNK)

The SNK method checks the error per comparison when comparing neighboring groups. As the groups Decouple from each other and the difference between the largest group average and the smallest group average is compared, the trial head checks the error. For this reason, the error per trial is checked in the SNK test (Zar, 1999; Özdamar 2004).

The Tukey Method

The method proposed by Tukey (1949) and called by his own name is based on a binary comparison of trial averages, as in the Duncan method. The Tukey method is based on Studentized range Statistics (Range Statistics) (Düzgüneş *et al*., 1987; Milliken and Johnson, 1992; Kesici and Kocabaş, 2007; Montgomery, 2008; Şenoğlu and Acıtaş, 2010; Genç and Soysal, 2018).

The Dunnet Method

It is a method used to compare the average of the treatment groups with a control group. The aim is to compare the means of the control group and the other groups. The Dunnet method, unlike other multiple comparison tests, can also be used in the analysis of variance even if the H_0 hypothesis has not been rejected (Zar, 1999; Özdamar, 2004).

The Scheffe Method

The method is used by Scheffe (1953) to test not only a small number of pre-planned linear connections, but also all possible connections (Zar, 1999; Kuehl, 2000; Montgomery, 2008; Genç and Soysal, 2018).

However, here it is useful not to use the LSD test when the number of groups is more than four. Because in this test, since the error per trial is not constant and changes with the number of treatments, even if the p value is taken as 0.05 when the number of groups is more than four, this is not really the p value. If small differences between groups are important for the researcher, the DUNCAN test, whose error is fixed per experiment, can be used to better decipher this. Researchers often prefer the DUNCAN test, which significantly Decouples a larger number of average differences relative to the SNK and TUKEY tests, which are slightly stricter tests, since they want to find, not find, a difference between the tested treatment averages.

Regression and Correlation Analysis

If range is the subject of two variables with a cause and effect relationship, then regression and correlation analysis, which will reveal the form and amount of this relationship, finds an application area.

In agriculture for example, there are cause-and-effect relationships between plot size and parcel yield, animal age and milk yield, plant height and yield, amount of fertilizer per Decare and yield. In these types of relationships, future outcomes can be predicted using prediction equations derived from a given set of available data (such as the prediction of lactation curves and growth curves in cattle) (Kayaalp and Bek, 1991; Efe, 1992).

Of course, the validity and correct choice of the regression model used when predicting the result are also important here. Whether this model fits well or not, and whether the assumptions made for the applied regression analysis method used hold for the data we are using, should also be checked by various statistical methods (Akar and Şahinler, 1993;1994). If not, it is always possible to enter some numbers into the computer and get some numerical results as a result. But this does not mean that that analysis is valid.

CONCLUSION

This study consists of a review of statistical methods used in veterinary science. The testing methods used in veterinary sciences are not limited and are being supported by new methods every day. As in all sciences, the importance of statistics in veterinary science is increasing day by day. It strengthens the accuracy of the statistical data and increases the reliability of scientific results. Before making statistics, it should definitely be decided by consulting a good statistician with which data, which results will be examined, and which test methods will be used. If this is not paid attention to, an incorrect test method or data pattern leads us to the wrong one.

Conflict of Interest

No conflict interest. All authors contributed to the study conception and design.

REFERENCES

Akar, M., & Şahinler, S. (1993). *İstatistik*. Ç.Ü.Ziraat Fakültesi Genel Yayın No: 74. Ders Kitapları Yayın No:17. Adana 362 Sayfa.

Akar, M., & Şahinler, S. (1994). Regresyonda Standart Kalıntılar ve Hatanın Normallik Varsayımının İncelenmesi. *ÇÜ Ziraat Fakültesi Dergisi*, *9*(2), 77-92.

Akbulut, Ö. (2024). Inferential Statistics Suggested to Report in Scientific Articles. KSU J. Agric Nat 27 (1), 238-247. DOI: 10.18016/ksutarimdoga.vi.1282850.

Bayir, T., & Tekin, M.E. (2022). Veteriner Bilimlerinde Yayınlanan Makalelerde Kullanılan İstatistiğin İncelenmesi. Veteriner Hekimler Derneği Dergisi, 93(1), 9-17

Bek,Y., & Efe, E. (1988). *Araştırma Ve Deneme Metodları I*. Ç.Ü. Ziraat Fakültesi Ders Kitabı, No: 71.395 Sayfa.

Büyüköztürk, Ş. (1997). İki faktörlü varyans analizi. *Ankara University Journal of Faculty of Educational Sciences (JFES)*, *30*(1).

Duncan, D. B. (1955). Multiple range and multiple F tests. *biometrics*, *11*(1), 1-42.

Düzgüneş, O., Kesici, T., Kavuncu, O., & Gürbüz, F. (1987). Araştırma ve deneme metotları. *Ankara Üniversitesi Ziraat Fakültesi Yayınları*, *1021*, 1-381.

Efe, E., Bek, Y., & Şahin, M. (2000). İstatistik Yöntemler II. *Kahramanmaras Sutcu Imam Unv., Bilgisayar Arastırma Ve Uygulama Merkezi, Publication*, (10).

Ervural, B. Ç. (2020). Varyans Analizi (Anova) Ve Kovaryans Analizi (Ancova) İle Deney Tasarımı: Bir Gıda İşletmesinin Tedarik Süresine Etki Eden Faktörlerin Belirlenmesi. *Bilecik Şeyh Edebali Üniversitesi Fen Bilimleri Dergisi*, *7*(2), 923-941.

Fisher, R. A. (1935). The logic of inductive inference. *Journal of the royal statistical society*, *98*(1), 39-82.

Gamgam, H., And Altunkaynak, B. (2008). Parametrik Olmayan Yöntemler SPSS Uygulamalı. Ankara: Gazi Kitabevi, pp.496

Genç, S., & Soysal, M. İ. (2018). Parametrik ve parametrik olmayan çoklu karşilaştirma testleri. *Black Sea Journal of Engineering and Science*, *1*(1), 18-27.

Kalayci, S. (2006). SPSS uygulamalı çok değişkenli istatistik teknikleri [SPSS applied multivariate statistical techniques]. *Ankara, Türkiye, Asil Yayın Dağıtım*

Karagöz, Y., Çatı, K., & Koçoğlu, C. M. (2015). Cep Telefonu Ve Operatör Tercihinde Etkili Olabilecek Faktörlerin Demografik Özelliklere Bağlı Olarak İrdelenmesi. *Dumlupınar Üniversitesi Sosyal Bilimler Dergisi*, (23).

Kayaalp, G. T., & Şahinler, S. (1996). İstatistiğin Tarımsal Araştırmalardaki Önemi Ve Kullanım Alanları. *Pikan Yetiştiriciliğinde Üzerinde Durulması Gereken Ekolojik Faktörler*, 59.

Keller G., and Warrack B. (2003), *Statistics for Management And Economics*, Brooks/Cole-Thomson Learning, USA.

Kesici, T., & Kocabaş, Z. (2007). Biyoistatistik. *Ankara Üniversitesi Eczacılık Fakültesi Yayın*, (94),

369.

Kuehl, R. O. (2000). Design of experiments: statistical principles of research design and analysis. *(No Title)*.

Milliken, G., & Johnson, D. (1992). Analysis of messy data: design of experiments.

Montgomery, D. C. (2017). *Design and analysis of experiments*. John wiley & sons.

Nie, N. H.(1976). *Spss Statistical Package for the Social Sciences*, vol. 5, no. 1. Informa UK Limited.

Özdamar K. 2004. Paket Programlar İle İstatistiksel Veri Analizi (Çok Değişkenli Analizler). Kaan Kitabevi, 528s, Eskişehir.

Ruxton, G., & Beauchamp, G. (2008). Some suggestions about appropriate use of the Kruskal–Wallis test. *Animal Behaviour*, *76*(3), 1083-1087.

Scheffé, H. (1953). A method for judging all contrasts in the analysis of variance. *Biometrika*, *40*(1-2), 87-110.

Soysal, İ. (2000). Biometrinin Prensipleri (İstatistik I ve II Ders Notları) TÜ Tekirdağ Ziraat Fak. *Yayınları. Yayın*, (95).

Şenoglu, B., & Acitas, S. (2010). istatistiksel deney tasarimi sabit etkili modeller. *Nobel, Ankara*.

Tukey, J. W. (1949). Comparing individual means in the analysis of variance. *Biometrics*, 99-114.

Zar, J. H. (1999). Biostatistical analysis. ed. *Princeton-Hall, New Jersey*.

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A Case of Proliferative and Necrotizing Otitis Externa in a Scottish Fold Shorthair Cat

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ABSTRACT

In this case report, the aim was to contribute to understanding PNOE's clinical presentation, histopathological features, and successful treatment approaches, emphasizing the significance of accurate diagnosis and appropriate management in affected felines. Proliferative necrotic otitis externa (PNOE) is a very rare skin disease of unknown etiology affecting the vertical ear canal and the concave surface of the auricle, which may be accompanied by secondary yeast or bacterial infections. In this case 8-month-old Scottish fold shorthair cat has fragile, papillomatous growths and caseous purulent exudate in the ear canal was presented. The differential diagnosis of this condition includes otoacariasis, plasma cell pododermatitis, papillomavirus and chronic bacterial or yeast otitis externa. Despite antimicrobial and antiparasitic based previous treatments, the lesions persisted. Histopathological examination revealed acanthotic epidermis, necrotic keratinocytes, and inflammatory cell infiltrates. Treatment involved methylprednisolone injection, topical pomades, and systemic antibiotics. The cat showed improvement after initial treatment week, with regression of lesions and cessation of ear discharge. Follow-up visits demonstrated continued improvement, and after two months, minimal sequelae remained. In conclusion, despite the unknown etiology of PNOE, the disease is quite responsive to immunosuppressive therapy, which is beneficial for both diagnosis and treatment.

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INTRODUCTION

Proliferative necrotic otitis externa (PNOE) is a very rare skin disease of unknown etiology affecting the vertical ear canal and the concave surface of the auricle, which may be accompanied by secondary yeast or bacterial infections (Mauldin *et al*., 2007; Borio *et al*., 2012; Momota *et al*., 2016). Plaque like lesions appear between 2 and 6 months of age in affected animals (Mauldin *et al*., 2007). Lesions may regress spontaneously within 12 to 24 months (Gross *et al*., 2008). The presence of scattered apoptotic keratinocytes within the highly hyperplastic epidermis and superficial follicular epithelium is the most prominent feature on histopathological examination. Luminal folliculitis is also frequent manifestation. Superficial and luminal pustulation and eosinophilic micro-abscesses of the epidermis have rarely been documented. (Vidémont and Pin, 2010). Amorphous debris, inflammatory cells and cocci can be found in the smear prepared from the exudate (Momota *et al*., 2016). The pathogenesis of the disease is still under investigation (Vidémont and Pin, 2010). Immunosuppressant drugs such as tacrolimus suppress inflammatory cells including T cells and inhibit keratinocyte hyperplasia by increasing cytokine production (Sanders *et al*., 2002). Alternatives to tacrolimus include clobetasol propionate cream and local injection of methylprednisolone acetate into the lesion (Momota *et al*., 2016). This disease, typically seen in kittens, should be considered among the differential diagnoses for bilateral otitis externa in young cats (Borio *et al*., 2012).

In this case report, the clinical symptoms, pathological findings and treatment approach of PNOE are aimed to present.

CASE HISTORY

Patient was presented to Van Yuzuncu Yil University, Animal hospital, Surgery department with the complaints of epiphora for 6 months and formation of fragile growths on the inner surface of the auricle for the last 1 month. The animal was an 8-month-old, unspayed female Scottish fold shorthair cat weighing 2.9 kg, with a white coat, never been in heat. According to the anamnesis, the cat had no previous allergic reaction to any substance and was fed with generic, pellet cat food by the owner. At least three times a week paste treats and wet food products were giving the cat. The lesions were first noticed by the owner as black dots like earwax and progressed rapidly and soon covered the entire inner surface of the auricle (Figure 1-A). During this process, owner observed loss of appetite and weakness. The cat had been treated for 21 days with a topical ear cleanser and an ear pomade containing neomycin, nystatin, triamcinolone acetonide, and permethrin, but no improvement in lesions was observed.

Clinical examination revealed that the lymph nodes were of normal size. Body temperature, heart rate, respiratory rate, and CRT were in normal range. It was observed that there were fragile, papillomatous and exudative growths on the concave surface of the pinna, and these growths continued until the entrance of the vertical ear canal. The external ear canal was partially filled with caseous purulent exudate, and when palpation was applied, the caseous contents came out. Otoscopic evaluation was not possible during the initial examination due to the lesions occluded the ear canal. Wood's lamp examination was negative for fungal infection.

Sampling was performed under anesthesia (0.1 mg/kg butorphanol, 0.04 mg/kg medetomidine, 1.5 mg/kg ketamine IM) for histopathological examination. In view of the clinical presentation, the diagnosis of proliferative necrotic otitis externa was established, and a methylprednisolone SC injection of 3 mg/kg was administered into the auricle during the same anesthetic session. Owner was advised to clean auricals with Crystalin® containing hypochlorous acid and then to apply thin layer of mixture of

Tacrolin® pomade and Furacin® pomade on the concave surface of the auricle 2 times. Systemically, Synulox® was prescribed for 7 days. Elizabeth collar was recommended to be used. It was recommended to switch from generic cat food to hypoallergenic commercial food and to reduce the frequency of wet food and treats for the diet.

For the complaint of epiphora, the obstruction of the nasolacrimal ducts was checked, and it was found normal. Conjunctivae were inflamed and there was serous discharge in the eyes. Tobradex® eye drops were prescribed every 4 hours for the first three days and every 6 hours for the following days.

Processing of samples

Specimens were processed routinely for fixation in 10% neutral buffered formalin, sectioning at 5 µm and staining with haematoxylin and eosin. In addition, Gram and PAS staining was carried out on the skin biopsies from extra-auricular lesions.

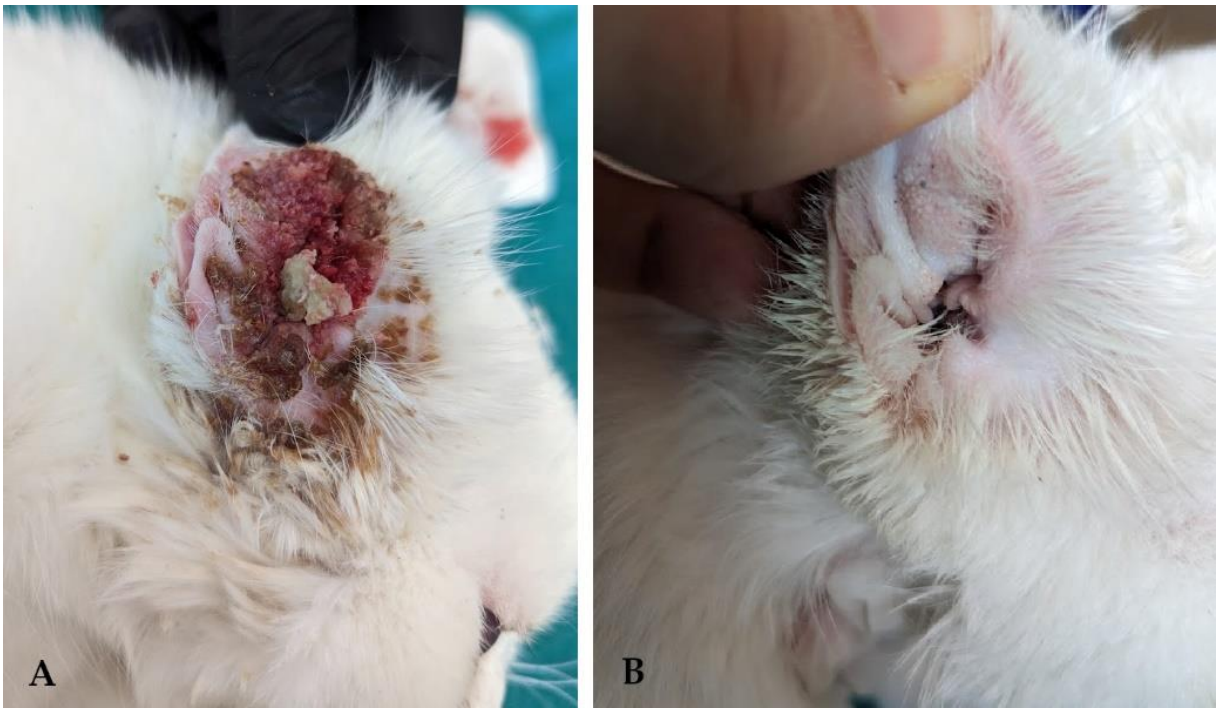

Figure 1. **A)** Proliferative formations in the inner surface of the pinna at the first evaluation. **B)** View of the inner pinna at two months after the initial treatment.

RESULTS AND DISCUSSION

Histopatologically, there were the small erosions and ulcers in the epidermis, and markedly acanthotic appearance in the epidermis and external root sheaths. The accumulations of cell debris were also observed on the acanthotic epidermis. Hyperplastic follicular external root sheath contained brightly eosinophilic and necrotic keratinocytes. In addition, the exocytosis of neutrophilic and lymphocytic cells from the dermis into the hyperplastic follicular external root sheath and numerous mitotic figures in keratinocytes of hyperplastic follicular sheaths noted. The inflammatory cell infiltrates consisted of plasma cells, lymphocytes, mast cells and neutrophils were observed in the dermis (Figure 2. A B C).

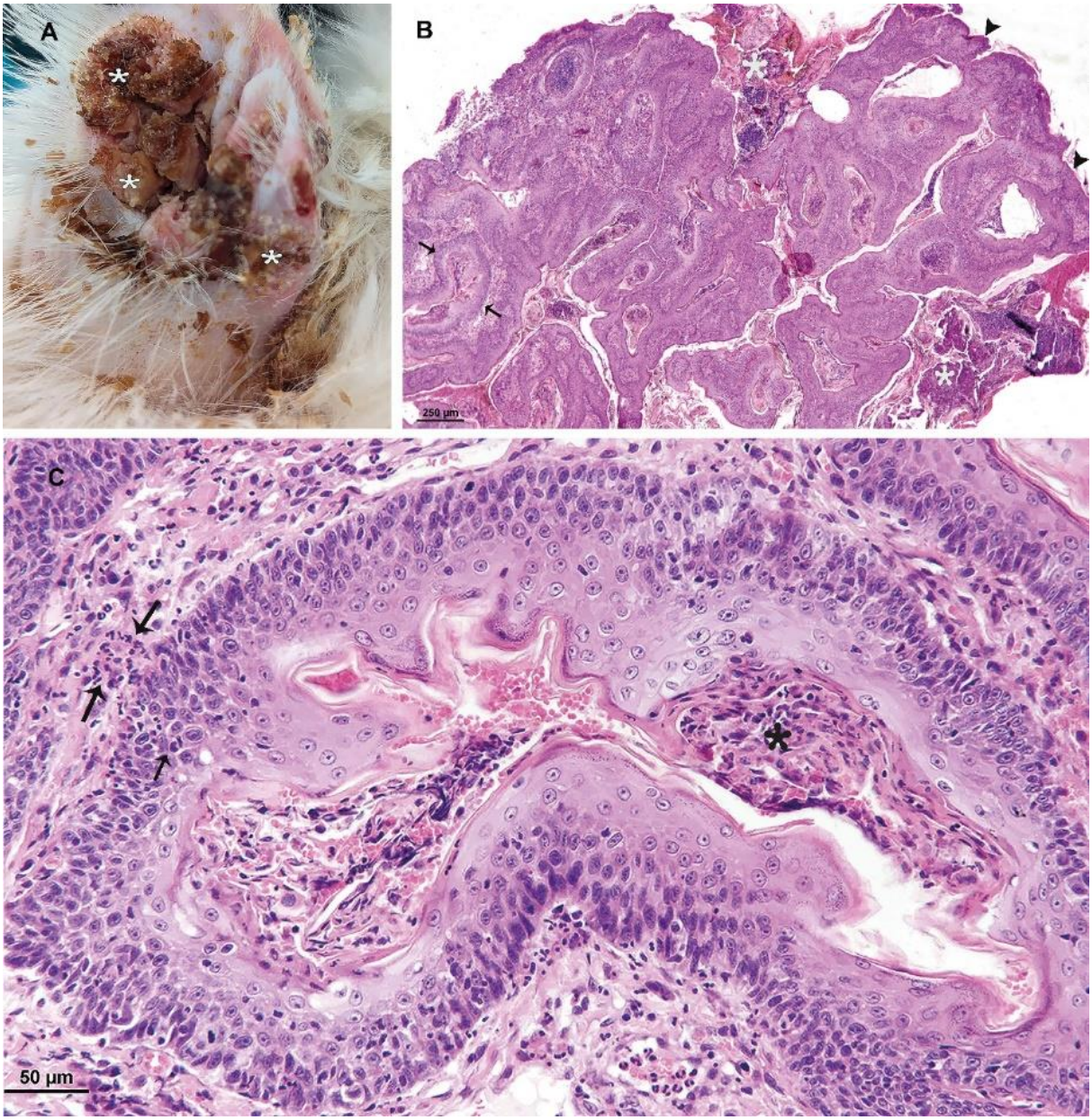

Figure 2. Feline proliferative and necrotizing otitis externa in a kitten with extensive extra-auricular formations.

(A) Shows the light brown to dark erythematous vegetative plaque with adherent keratinous debris lining the inner part of the pinna and the canal (*). There are also small erosions or ulcers on the inner part of the auricle.

(B) Showing markedly acanthotic appearance the epidermis (arrowheads) and external root sheaths (arrows). Accumulation of cell debris (*) is observed on the acanthotic epidermis. (C) Higher magnification of the acanthotic root sheath between the arrows in picture B. Showing brightly eosinophilic and necrotic keratinocytes (parakeratotic hyperkeratosis) (*) the exocytosis of neutrophilic and lymphocytic cells from the dermis into the hyperplastic follicular outer root sheath (arrows).

At the first control, after the 1 week of initial treatment, it was determined that the discharge from the ear canal had stopped and the proliferative lesions on the concave surface of the auricle had regressed. It was also learned that the pomades caused itching in the ears. The appetite was increased.

Hemogram and biochemistry values of the blood samples were found to be normal.

Two weeks after the first examination, the use of Furacin® pomade was discontinued because the exudation and secondary infection in the lesions disappeared. During the follow-up visits of one-week intervals, the lesions gradually regressed, and the general condition improved. About one month after the first visit, 99% of the lesions had disappeared and otoscopic examination showed no growths in the ear canal. Tacrolin® pomade and Crystalin® spray were recommended for 2 more weeks. After 2 months, it was determined that minimal sequelae (blackheads) remained on the auricle (Figure 2).

Plasma cell pododermatitis, papilloma virus, and fungal infections are important in the differential diagnosis of the disease. Papillomavirus (PV) is a causative agent of hyperplastic growths in cats (Munday and Thomson, 2021). Progression of the PV-associated lesions suggest a cause, such as stress, and it should be kept in mind that glucocorticoids may trigger virus expression and enhance the development of use-associated lesions. Since exposure to PV or other viral agents causes increased sensitization, topical and oral glucocorticoid use is generally contraindicated (Nagata and Rosenkrantz, 2013).

In our case, both topical application of tacrolimus pomade and local prednisolone injections were performed. In contrast, a very rapid regression of the lesions was observed.

Plasma cell pododermatitis is a disease of uncertain etiology that causes lesions in the ears and pulvinus and usually responds to immunomodulatory treatment (Brosseau, 2022).

In our case, although there were lesions in the ears, there were no lesions in the pulvinus, and it responded to treatment based on the principle of immunosuppression.

Wood's lamp is a source of long wave ultraviolet light and has diagnostic utility in detecting fluorescence in skin and hair, a characteristic of some dermatophytes in infected skin and hair that is invisible to the naked eye (Al Aboud and Gossman, 2022). It is differentiated from fungal infection by the fact that the lesions were confined to the ear and the Wood's lamp test was negative.

It has been noted that the main diagnostic microscopic lesions of proliferative otitis externa are characterized by papillomatous hyperplasia and follicular epidermal hyperplasia with hyperkeratosis and parakeratotic hyperkeratosis of the epidermis, necrotic neutrophilic crusting covering the epidermal surface and obstructing the hair follicle ducts. It was also reported that single cell necrosis/apoptosis in different layers of the epidermis, marked lymphocytic exocytosis and satellitosis of dead keratinocytes. In addition, it has been noted that in the dermis, depending on the duration of inflammation, inflammatory cell infiltrates typically consist of plasma cells, lymphocytes, mast cells and neutrophils (Mauldin *et al*., 2007; Brian & Bradley, 2015; Momota *et al*., 2016; Panzuti *et al*., 2021). The histopathological findings of presented herein was very similar for those described by Mauldin et al., 2007; Momota *et al*., 2017; Panzuti *et al*., 2021. However, inflammatory cell infiltrations in the dermis were differently observed to consist of lymphocytes and neutrophils. Based on these characteristic histopathological findings, presented herein a diagnosis of proliferative and necrotizing otitis was made.

In conclusion, despite the unknown etiology of PNOE, the response of the disease to immunosuppressive therapy is quite remarkable. This situation contributes to both the diagnosis and treatment of the disease.

Ethical Approval

This manuscript is a case report. It is not required any institutional ethics approval for case reports. We have informed consent document from the owner.

Conflict of Interest

Authors have no conflict of interest to declare. All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Caner KAYIKCI, Ömer Faruk KELEŞ, Yağmur KUŞCU, Selime Nur EKICI and Hacı Ahmet ÇİÇEK. The first draft of the manuscript was written by Caner KAYIKCI and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

REFERENCES

Al Aboud, D.M., & Gossman, W. (2022), Wood's Light, StatPearls Publishing, Treasure Island (FL).

Borio, S., Massari, F., Abramo, F., & Colombo, S. (2012), Proliferative and necrotising otitis externa in a cat without pinnal involvement: video-otoscopic features. *Journal of Feline Medicine and Surgery 15*(4), 353–356.

Brian, P.W. & Bradley, L.N. (2015), Special Senses. In: Jubb KVF, Kennedy PC and Palmer N (eds.), Pathology of domestic animals, vol. 1, 6th edition, Academic Press, London, 502–503.

Brosseau, G. (2022), Feline plasma cell pododermatitis. *The Canadian Veterinary Journal 63*(5), 545.

Gross, T.L., Ihrke, P.J., Walder, E.J., & Affolter, V.K. (2008), Necrotizing diseases of the epidermis, Skin diseases of the dog and cat: clinical and histopathologic diagnosis, John Wiley & Sons, 79–91.

Mauldin, E.A., Ness, T.A., & Goldschmidt, M.H. (2007), Proliferative and necrotizing otitis externa in four cats. *Veterinary Dermatology, 18*(5), 370–377.

Momota, Y., Yasuda, J., Ikezawa, M., *et al*. (2016), Proliferative and necrotizing otitis externa in a kitten: successful treatment with intralesional and topical corticosteroid therapy. *Journal of Veterinary Medical Science, 78*(12), 1883-1885.

Munday, J.S., & Thomson, N.A. (2021), Papillomaviruses in domestic cats. *Viruses, 13*(8), 1664.

Nagata, M., & Rosenkrantz, W. (2013), Cutaneous viral dermatoses in dogs and cats. Compendium (Yardley, PA) 35 (7), E1–E1.

Panzuti, P., Jongh, O., Dony, M., Vial, S., Mosca, M., & Pin, D. (2021), Extra-auricular lesions of proliferative and necrotizing otitis externa in three kittens. *Veterinary Dermatology 32*(4), 385-e110.

Sanders, S., Busam, K.J., Halpern, A.C., & Nehal, K.S. (2002), Intralesional Corticosteroid Treatment of Multiple Eruptive Keratoacanthomas: Case Report and Review of a Controversial Therapy, *Dermatologic Surgery 28*(10).

Vidémont, E., & Pin, D. (2010), Proliferative and necrotising otitis in a kitten: first demonstration of T-cell-mediated apoptosis. *Journal of Small Animal Practice 51*(11), 599–603.