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HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Harran University
Journal of the Faculty of Veterinary Medicine

Harran Üniversitesi Veteriner Fakültesi Yayınıdır
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HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Geometric Morphometric Analyses on Radiographic Images of The Carpal Joint Region of English Setters

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Abstract: This study aims to determine the morphological differences according to gender via radiographic images taken from the carpal bones of English setters. A total of 28 bone samples, 16 females and 12 males, were used in the study. The shape differences between males and females were evaluated by placing 27 landmarks on the X-ray images. Principal Component Analyses are used to measure the distribution of variance. In addition, these analyses take the mean shape variation and compare it to shape differences. As a result of this analysis, the value of PC1 explained 24.34% of the total variation. In addition, PC2 explained 16.24% of the total variation, and PC3 explained 10.45%. In addition, the results of PCA were also described in terms of the English setter samples used. Then, Discriminant Function analysis was performed using the same software program. With this analysis, it was tried to determine the differences between the genders. According to the discriminant function test, 13 of 16 female dogs were completely separated from male dogs. Also, all 12 male dogs were separated from female dogs according to the same test results. In short, because of this study, shape differences in the carpal bones of English setters were investigated according to gender.

Keywords: *Carpal joint, English Setters, Geometric morphometry, Radiography, Veterinary anatomy.*

İngiliz Setter Köpeklerinin Karpal Eklem Bölgesinin Radyografik Görüntüleri Üzerinde Geometrik Morfometrik Analizler

Özet: Bu çalışmada, İngiliz setter köpeklerinin karpal kemiklerinden alınan radyografik görüntüler aracılığıyla cinsiyete göre morfolojik farklılıkların belirlenmesi amaçlanmıştır. Çalışmada 16 dişi ve 12 erkek olmak üzere toplam 28 kemik örneği kullanılmıştır. Erkek ve dişiler arasındaki şekil farklılıkları, röntgen görüntüleri üzerinde toplam 27 adet işaretleme ile değerlendirilmiştir. Varyans dağılımını ölçmek için Principal Component Analysis (PCA) kullanılmıştır. Bu analizlerin kullanım amacı ortalama şekil varyasyonunu alıp şekil farklılıklarıyla karşılaştırmaktadır. Bu analiz sonucunda PC1 değeri toplam varyasyonun %24,34'ünü açıklamıştır. Ayrıca PC2 toplam varyasyonun %16,24'ünü, PC3 ise toplam varyasyonun %10,45'ini açıklamıştır. Daha sonra örnekler Diskriminant Fonksiyon analizi üzerinden de test edilmiştir. Bu analiz ile cinsiyetler arasındaki farklılıklar belirlenmeye çalışılmıştır. Diskriminant fonksiyon testine göre 16 dişi köpeğin 13'ü erkek köpeklerden tamamen ayrılmıştır. Yine aynı testin sonuçlarına göre 12 erkek köpeğin tamamı dişi köpeklerden ayrılmıştır. Kısacası, bu çalışma sonucunda İngiliz setter köpeklerinin karpal kemiklerindeki şekil farklılıkları ve cinsiyete göre karpal kemiklerde oluşan değişimler araştırılmıştır.

Anahtar Kelimeler: *Geometrik morfometri, İngiliz Setter, Karpal kemikler, Radyografi, Veteriner anatomi.*

Introduction

The setter dog breeds are classified into seven groups, including 153 dog breeds determined by the American Kennel Club (AKC) (Evans and De Lahunta, 2012). These seven groups identified by the American Kennel Club (AKC) show genetic diversity as well as anatomically (Evans and De Lahunta, 2012; Irion et al., 2003). According to the American Kennel Club (AKC), these dog breeds are in the sporting dog group. Also, this dog breed is thought to have English ancestry (Evans and De Lahunta, 2012). In this study, the samples taken from the carpal bones of the setter dog breed were used. The setter dog breed has a broad scale including various dog types such as a pointer, Irish setter, British setter, and red setter. This dog breed has many uses, such as hunting, farm work, and show. For example, pointer setters are mainly used for hunting, and Irish setters are primarily used for the show. It is observed that English setters are used in almost every field (Pedersen et al., 2013). Also, according to the Norwegian Kennel Club, English Setters are one of the athletic dog breeds. This dog breed resists long- distance walks and rough terrain (Vatne et al., 2021). For this reason, it is aimed to examine the anatomical examination of such a mobile and athletic dog breed and to discuss the differences in the bones.

The carpal bones, or as it is named in veterinary anatomy, "*ossa carpi*", formed the first part of the forefoot bones (*skeleton manus*). The carpal bones compose eight bones in all domestic mammals, *proximal* and *distal* rows. These were named *os carpi radiale, os carpi intermedium, os carpi ulnare*, and *os carpi accessorium* from *medial* to *lateral* on the *proximal* row. The carpal bones on the distal side are called *os carpale 1, os carpale 2, os carpale 3*, and *os carpale 4*. The carpal bones of dogs differ in number and structure from those of other domestic mammals. In these animals, the carpal bones on the proximal row are 3 instead of 4. The bones of the *os carpi radiale* and the *os carpi intermedium* fused to form the *os carpi intermedioradiale*. In addition to these bones, *os carpi ulnare* and *os carpi accessorium* are also found in these animals. The dogs have all four carpal bones in the *distal* row. The articulations of the carpal bones are named as follows. The joint between the *antebrachium* and the carpal bones is called the *Articulatio antebrachiocarpea*. The articulations between the upper and lower rows of the carpal bones are called *Articulationes mediocarpea*. The *Articulationes intercarpea* are the joints made by the carpal bones located on the same row. The articulations of the carpal bones with the metacarpal bones are called *Articulationes carpometacarpea* (Demiraslan and Dayan, 2021; Dursun, 2007; König et al., 2007; Nomina Anatomica, 2017).

Geometric morphometry is a statistical analysis type based on the shape analysis of the structure, revealing shape variations for groups. This analysis, frequently used in veterinary anatomy in recent years, enables gender analysis and taxonomy studies. In studies on skulls, gender, and breed analysis can be performed in birds, civets, and dogs (Jashari et al., 2022; Gundemir et al., 2023a; Gundemir et al., 2020b; Gundemir et al., 2023b; Gurbuz et al., 2022; Szara et al., 2022). It has been reported that using geometric morphometry and gender analysis is effective in studies involving geometric shapes, such as the turtle's carapace layer (Duro et al., 2021). Gender analysis was investigated by applying geometric morphometry to the finger postures of birds (Demircioglu et al., 2022).

Fractures in the carpal bones can be observed frequently, and this may vary with variables such as the animal's mobility capacity, age, breed, and weight (Li et al., 2000; Rainbow et al., 2008). However, there is limited knowledge about the anatomical differences between the sexes of the carpal joints in carnivores. It was estimated that there were linear differences between the articular bones regarding gender in cats.

The previous studies revealed the differences between genders between joint measurements or shapes (Gundemir et al., 2021; Şenol et al., 2022). This study aimed to examine the carpal arrangement of the English setter dog species by geometric morphometry method and to reveal the difference between males and females in shape.

Material and Methods

Ethical Statement

Permission was obtained from the Ethics Committee of the Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa, to use the samples and conduct the study (Decision number: 2022/38)

Animals and radiographs

This study used 28 (16 female and 12 male dogs) English setters. Radiographic images of the right carpal bones of English setter dogs were used. The carpal bones of all dogs whose radiographic images were collected were healthy. No pathological findings were found in the images used. The female dogs used in the study were 1-6 years old and weighed 14-30 kg, and the male dogs were 1-8 years old and weighed 13-30 kg. Radiographic images were obtained from Istanbul University-Cerrahpaşa. Analysis was performed only on the dorso-palmar aspect of the right carpal bone. In addition, the distal of the radius and ulna bones and the proximal of the metacarpal bones were also visualized.

Radiographic landmarks and the abbreviations used in this Study

The abbreviations used throughout this study and their meanings are given in Table 1. The locations of the abbreviations are shown in Figure 1 on the radiographic image. The places where the landmarks are marked are shown on the radiographic image in Figure 2. In addition, the meanings of the landmarks are explained as follows.

L1-L2: Both landmarks were located at the distal end of the *ulna* bone. It is marked to form the two furthest points of the *processus styloideus lateralis*.

L3-L4: Both landmarks were located at the distal end of the radius bone. A landmark was marked on the *trochlea radii* (L3). The other landmark was marked on the *processus styloideus medialis* (L4).

Table 1. Representation of the abbreviations used in this study.

Abbreviation	Places	Landmarks Placed on Bones
U	Ulna	L1-L2
R	Radius	L3-L4
CU	Os carpi ulnare	L5-L6-L7-L8
CR	Os carpi intermedioradiale	L9-L10-L11-L12
C1	Os carpi 1	L22-L23-L24-L25
C2	Os carpi 2	L18-L19-L20-L21
C3	Os carpi 3	L13-L14-L15-L16-L17
MC	Metacarpus	L26-L27

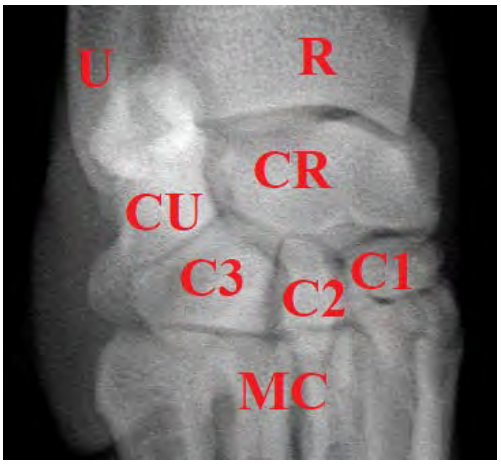

Figure 1. Representation of the abbreviations used in this study on the dorsopalmar radiographic image (U: Ulna, R: Radius, CU: Os carpi ulnare, CR: Os carpi intermedioradiale, C1: Os carpi 1, C2: Os carpi 2, C3: Os carpi 3, MC: Metacarpus)

Figure 2. Dorsopalmar radiographic image of *carpal* bones showing location of landmarks (LM).

L5-L6-L7-L8: Each of the four landmarks were marked on the *os carpi ulnare*.

L9-L10-L11-L12: Each of the four landmarks were marked on the corners of the *os carpi intermedioradiale*.

L13-L14-L15-L16-L17: Each of the five landmarks were marked on the corners of *os carpi 3*.

L18-L19-L20-L21: Each of the four landmarks were marked on the corners of *os carpi 2*.

L22-L23-L24-L25: Each of the four landmarks were marked on the corners of *os carpi 1*.

L26-L27: Landmarks are marked as the furthest distance between the medial and lateral sides of the proximal end of the metacarpal bones visible on the radiographic image.

Geometric morphometry

The images of the carpal bones of the dogs taken from the X-ray device were converted to Jpeg format and transferred to the computer. The images in Jpeg format were converted to TPS files using the TPS Util (version 1.82) program. Then, they were marked on the images using the TPS Dig2 (version 2.31) program. All veterinary anatomy terms used in this study are taken from Nomina Anatomica Veterinaria (Nomina Anatomica Veterinaria, 2017).

Radiographic images were the view of the right carpal bones from the dorsopalmar position. The images also showed the distal section of the antebrachium bone (Radius-Ulna) and the proximal section of the metacarpal bones. These bones, which appear in the images, are also included in the markings.

Statistical analysis

In this study, shape analyses were performed using the MorphoJ (version 1.07a) software (Klingenberg, 2011). The carpal bones were grouped by gender, and then the differences between the sexes were investigated with the help of this software. The landmarks indicate anatomically specific points (Bookstein et al.,1991). Using this software, coordinate data were tested morphologically, and these data were tried to be interpreted in terms of geometric morphometry. Procrustes Fit was first applied to all the samples embedded in the software, and then Generalized Procrustes Analysis and Principal Component Analysis (PCA) were applied. The purpose of Procrustes Analysis is to display shape differences. Generalized Procrustes Analysis is used to reveal general shape differences. Principal Component Analysis (PCA) is used to measure variance distribution. The PCA is a coordination method that aims to reduce the dimensionality of the data from the samples. Discriminant Function analysis was applied to all groups to investigate the differences between the sexes. With this method, it was aimed to find variations between females and males.

Results

The results of five different principal component values of these landmarks determined according to the MorphoJ program are given in Table 2. According to this table, PC1 explained 24.34%, PC2 16.24%, and PC3 10.45% of the total variance.

Shape variation of PC values were given in Figure 3 with the Wire-frame warp plots. The placement of the PC values in the Variance Graph is shown in Figure 4. The blue frame represents the mean shape of all samples, and the red frame represents the positive limit. The red frames were compared with the blue frames, as the blue frames gave the mean shape.

A sharp curve was formed between LM2 and LM3, which remained between U and R at PC1 value. Carpal bones were evaluated according to the PC1 value. Accordingly, it

Table 2. Results of principal component analysis.

Principal Components	Eigenvalues	Variance %	Cumulative %
PC1	0,00152116	24,345	24,345
PC2	0,00101482	16,241	40,586
PC3	0,00065337	10,457	51,043
PC4	0,00052995	8,481	59,524
PC5	0,00045782	7,327	66,852

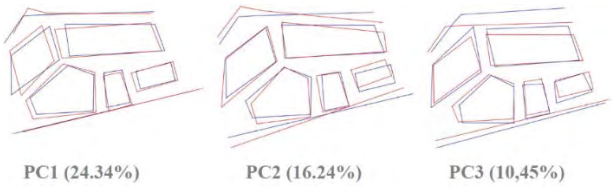

Figure 3. Variance Distribution for Principal Components. Blue outlines represent the mean shape configuration, while the red outlines show the shape changes associated with the positive extremes of the PC axes.

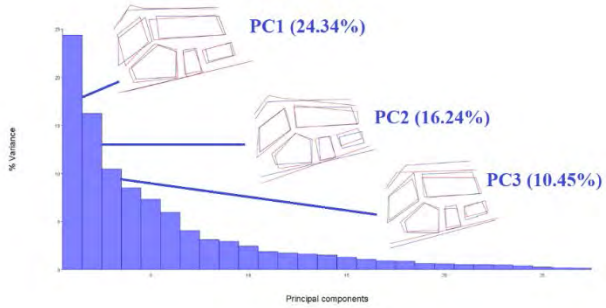

Figure 4. Representation of Principal Components on the variance graph. Blue outlines represent the mean shape configuration, while the red outlines show the shape changes associated with the positive extremes of the PC axes.

was seen that the CU was narrower but longer. The positive limit in the CR was transverse. In the lower row of carpal bones, the positive limit of C3 was observed to be both transverse and longitudinal. C2 followed a course closer to MC and showed a wider shape as it approached MC. Although C1 was almost the same size as the mean shape, it was wider than the mean shape. The landmarks on the MC were almost on the same course as the mean shape.

At PC2, landmarks at U and R showed a line that would cross the mean shape. Likewise, landmarks placed in the MC also crossed the mean shape. The edge where the LM7 was placed in the CU showed a sharper shape. However, other positive changes were almost identical to the mean shape. A positive limit was observed in the CR, which started broadly and narrowed horizontally. The positive limit of C3 was observed to be longer but narrower than the mean shape. C2 had a wider positive limit than the mean shape. The positive limit of C1 was located further upstream from the MC compared to the mean shape.

When the PC3 value was examined in general, it was observed that the positive limits of the bones were flatter than the mean shape. U and R are lower in PC3 than the mean shape. The PC3 value represented a smaller CU value. The positive change in CR was more transverse but shorter in width. PC3 value was higher in all carpal bones in the lower row. It showed a wider shape in C3. It was narrower in shape in C2 and wider in C1. In MC, the positive change was located higher up.

The graphs of gender distribution in carpal bones depending on PC values are given in Figure 5. The red dots in these graphs represent female dogs, and the blue dots represent male dogs. Ellipses represent 95% confidence intervals around the means for sexes. Accordingly, less variation was observed in females. Male dogs showed more variation compared to females for both graphs.

According to the graph of PC1 and PC2, the highest PC1 value was measured to be the female sample. However, the highest PC2 value was observed in the male sample. According to the graph of PC1 and PC3, it was observed that the highest PC3 value was in the male sample. As a result, when all the graphics related to PC values were evaluated, no regional distribution was observed between female and male individuals. Female specimens and male specimens are scattered all over the graph.

The distinction of male and female individuals according to discriminant function analysis is given in Table 3. In addition, the discriminant scores, and the frequency graph, which were also shaped according to this analysis, are shown in Figure 6. According to these data, all 12 male samples were separated from females. In females, 13 out of 16 female samples were separated from males, while 3 of them did not show complete discrimination. However, according to Figure 6, an evident distance was observed between female and male samples. The differences between the sexes are shown in Figure 7 with the Wire-frame Wrap plot. In this graph, the mean values of female dogs are represented by a red frame and that of male dogs by a blue frame.

Table 3. Results of discriminant function analysis.

	Female	Male	Total
Female	13	3	16
Male	0	12	12

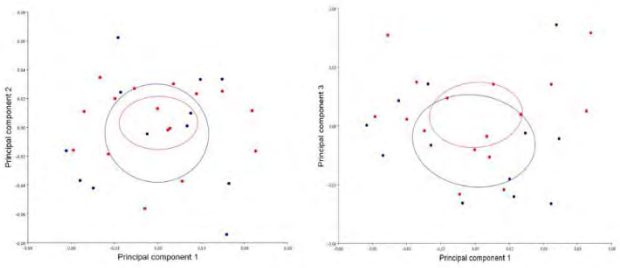

Figure 5. The Gender Distribution plot of carpal bones by the PC1 (24.34 %), PC2 (16.24 %) and PC3 (10.45 %). Ellipses represent 95% confidence intervals around the means for sexes (Male: blue; Female: red).

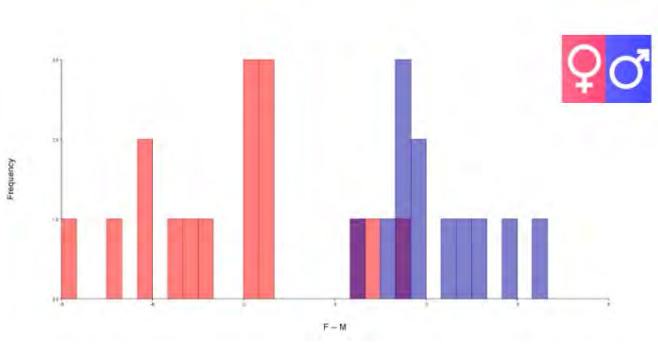

Figure 6. Discriminant scores and frequency for setter dogs carpal bones according to gender (Male: blue; Female: red).

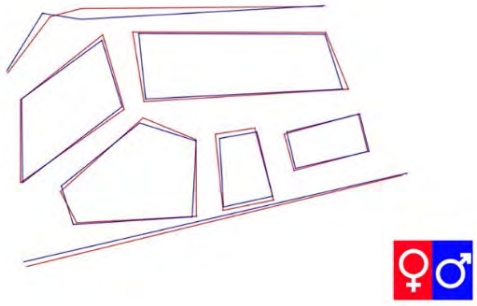

Figure 7. For female and male dogs carpal bones mean shape and difference between means (Male: blue; Female: red).

The frame between the signs on the U and R formed a sharper shape in males. On the contrary, it has been observed that female individuals form a more trapezoidal shape. When the carpal bones in the upper row were examined; CU was wider in female dogs. It was observed that the CR was horizontal or even shorter in males than in females. When carpal bones in the lower row were examined, C3 was wider in females. Only at the LM17 point the C3 of females was narrower than that of males. C2 showed a shorter and smaller shape in males. C1 was almost the same size in males and females, only at LM25 and LM22 points C1 of females was slightly larger in shape. Two landmarks were placed on the MC (LM26-LM27). While LM26 was separated into females and males, such a distinction was not observed in LM27. It was observed that the discrimination decreased as it approached the LM27 linearly.

The distance between the joint spaces between the carpal bones was greater in males. (*Articulationes mediocarpea* and *Articulationes intercarpea*). However, the distance between the upper carpal bones' U and R articular spaces was closer (*Articulatio antebrachiocarpea*). Likewise, the spacing of the joints of the lower-order carpal bones with the MC was narrower in males (*Articulationes carpometacarpea*).

Discussion and Conclusion

This study aimed to investigate gender differences in carpal bones. The development of morphological and anatomical studies needs to examine whether there is a distinction between carpal bones in female dogs and male dogs in English setters. It is also crucial for forensic medicine studies to determine whether these gender-related differences are specific to races. Such studies, it is aimed to increase the bones that can be evaluated instead of missing bones (Mastrangelo et al., 2011b). The diversity of these bones also provides the development of taxonomy (Bidmos-Steinberg et al., 2005; Introna Jr. et al., 1998; Giurazza et al., 2013; Gundemir et al., 2020a). For this purpose, radiographic images of carpal bones were taken from 28 English setter dogs. Twenty-seven landmarks were placed on these images. Principal Component Analysis (PCA) and Discriminant Function tests were applied to the visuals prepared. According to the result of PCA, the PC1 value explained 24.34% of the total variance.PC2 value explained 16.24% of the total variance. The PC3 value explained 10.45% of the total variance. Figure 5 shows the confidence intervals of both female and male individuals. According to this, female dogs showed a more conservative shape variation compared to male dogs.

According to discriminant function test results, all 12 male English setters were separated from the females. On the other hand, 13 out of 16 female English setters were completely separated from males, while 3 female English setters were not wholly separated from males. It was previously stated that the setter dogs whose images were taken in the study did not have any orthopedic disorders. In addition, the animals were selected from a certain weight and age range. For this reason, the three female English setters could not show complete separation from the male group, which may be caused by ossification (Myo et al., 2016).

Similar principal component values were found in another animal study on carpal bones. This study was done on horses. However, the comparison results between mean value and shape changes are close to this study. It was also studied on carpal bones as used in this study. In that study, the value of PC1 was 28.44%, also, the PC1 value was the highest variance percentage (Gundemir et al., 2021). The variation percentages were high when the principal component values were evaluated in this study. It was observed that the principal component value with the highest variance percentage was PC1 (24.34%) (Gundemir et al., 2021).

All 12 male dogs were completely separated from the female dogs in shape, while 3 out of 16 female dogs were not separated from the male dogs. Considering that samples from English Setter dog breeds were used in this study, the results may have been obtained this way (Evans et al., 2013; González-Rellán et al., 2022; Irion et al., 2003). It has also been reported that setters are used for tasks that rely on the strength of the musculoskeletal system, such as hunting and farm work, and for more comfortable purposes, such as dog shows (Evans et al., 2013; Irion et al., 2003). In other animals, it has been observed that the change in purpose of use can cause many problems and differences in the skeletal system (Bidmos et al., 2005; Li et al., 2000). In addition, the ages of the English setter specimens used in this study were

different from each other. This age difference may have affected bone development and size (Riggs et al., 2004). This may be the reason why 3 samples from female dogs were not separated from male dogs. Discriminant analysis has been previously applied to radiographic images and various bone measurements. This method has been applied to measurements of human and animal bones. It has also been used for gender dimorphism (Barnes et al., 2020; Gundemir et al., 2021; Mastrangelo, et al., 2011a; Mastrangelo et al., 2011b). In addition, the MorphoJ software program, used as a geometric morphometric method in this study, has been used for sex determination in other studies (Sarač-Hadžihalilović et al., 2022).

This study used radiographic images of the right carpal bones of 28 different setter dogs. The obtained images were transferred to MorphoJ software and analyzed in terms of change according to gender. For this purpose, 27 landmarks were added to all images. These landmarks were generally placed by targeting the end corners of the bones observed in the images. Principal component values were measured using the MorphoJ software system. It was tried to explain the visible changes according to the comparison results. In addition, the difference between male and female individuals was measured with the discriminant function test. The gender-related differences in the carpal bones in the setter dog breed were questioned. According to test results, landmark locations differed in male and female dogs (Figure 6). They were also separated from female dog samples of all 12 male dog samples. However, according to the test results, 3 out of 16 female dog samples did not differentiate from male individuals (Table 3).

Conflict of Interest

The authors stated that they did not have any real, potential, or perceived conflict of interest.

Ethical Approval

Permission was obtained from the Ethics Committee of the Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa, to use the samples and conduct the study (Decision number: 2022/38)

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Data Availability

Any additional data supporting this study are available from the author (E.O.) upon reasonable request

Author Contributions

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Design: YA, EO, EÖ, GP
Control/Supervision: YA, EÖ, GP
Data Collection and / or Processing: YA
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Writing the Article: EO

References

Barnes AE, Case DT, Burnett SE, Mahakkanukrauh P, 2020: Sex estimation from the carpal bones in a Thai population. *Aust J Forensic Sci,* 52 (6), 665-680.

Bidmos MA, Steinberg N, Kuykendall KL, 2005: Patella measurements of South African whites as sex assessors. *Homo*, 56 (1), 69-74.

Bookstein FL, Grayson B, Cutting CB, Kim HC, McCarthy JG, 1991: Landmarks in three dimensions: reconstruction from cephalograms versus direct observation. *Am J Orthod Dentofacial Orthop*, 100 (2), 133-140.

Demiraslan Y, Dayan MO, 2021: Veteriner Sistemik Anatomi. 1st ed., Nobel Tıp Kitabevleri, Ankara, Türkiye.

Demircioglu I, Duro S, Gungoren G, Choudhary OP, Gundemir O, Demiraslan Y, Pazvant G, 2022**:** Digits angle and digits length ratio in japanese quail (Coturnix coturnix japonica). *Indian J Anim Res*, 56 (9), 1105-1109.

Duro S, Gundemir O, Sönmez B, Jashari T, Szara T, Pazvant G, Kambo A, 2021: A different perspective on sex dimorphism in the adult Hermann's tortoise: geometric morphometry. *Zool Stud*, 60: e9.

Dursun N, 2007: Veteriner Anatomi 1, Medisan Yayınevi, Ankara, Türkiye.

Evans HE, De Lahunta A, 2012**:** Miller's anatomy of the dog-E-Book. 4th ed., Elsevier health sciences, Missouri, MO, USA.

Giurazza F, Schena E, Del Vescovo R, Cazzato RL, Mortato L, Saccomandi P, Zobel BB, 2013: Sex determination from scapular length measurements by CT scans images in a Caucasian population. In 2013 35th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, (EMBC) (pp. 1632-1635).

González-Rellán S, Barreiro A, Cifuentes JM, Fdz-de-Trocóniz P, 2022: Anatomy of the PaLar Region of the Carpus of the Dog. *Animals*, 12 (12), 1573.

Gundemir MG, Szara T, Spataru C, Demircioglu I, Turek B, Petrovas G, Spataru MC, 2023a: Shape differences of the Carina sterni in birds of various locomotion types. *Anat Histol Embryol,* 52 (2), 190-196.

Gundemir O, 2022: Skull of the Asian (Paradoxurus Hermaphroditus) and the golden (Paradoxurus Zeylonensis) paL civet: Geometric morphometric analysis using palate, tooth and frontal landmarks. *Anat Histol Embryol,* 51 (6), 718-727.

Gundemir O, Duro S, Jashari T, Kahvecioglu O, Demircioglu İ, Mehmeti H, 2020a: A study on morphology and morphometric parameters on skull of the Bardhoka autochthonous sheep breed in Kosovo. *Anat Histol Embryol,* 49 (3), 365-371.

Gundemir O, Hadžiomerović N, Pazvant G, Erdikmen DO, 2021**:** Radiometric and geometric morphometric analysis of the carpal joint area in 2-year-old Thoroughbred horses. *Veterinaria,* 70 (2), 209-217.

Gundemir O, Özkan E, Dayan MO, Aydogdu S, 2020b: Sexual analysis in turkey (Meleagris gallopavo) neurocranium using

geometricmorphometric methods. *Turk J Vet Anim Sci,* 44 (3), 681-687.

Gundemir O, Szara T, Yalin EE, Karabagli M, Mutlu Z, Yilmaz O, Parés-Casanova PM, 2023b: Examination of Shape Variation of the Skull in British Shorthair, Scottish Fold, and Van Cats. *Animals,* 13 (4), 614.

Gurbuz İ, Demiraslan Y, Rajapakse C, Weerakoon DK, Fernando S, Spataru MC, Gundemir O, 2022: Skull of the Asian (Paradoxurus Hermaphroditus) and the golden (Paradoxurus Zeylonensis) palm civet: Geometric morphometric analysis using palate, tooth and frontal landmarks *Anat Histol Embryol,* 51 (6), 718-727.

Horst Erich König, Hans-Georg Liebich, 2007: Veterinary Anatomy of Domestic Mammals. 3rd ed., Schattauer, Stuttgart, Germany.

International Committee on Veterinary Gross Anatomical Nomenclature (I.C.V.G.A.N.), 2017: Nomina Anatomica Veterinaria, 6th ed., Published by the Editorial Committee, Hanover, Germany, Ghent, Belgium, Columbia, MO, USA, Rio de Janeiro, Brazil.

Introna Jr F, Di Vella G, Campobasso CP, 1998: Sex determination by discriminant analysis of patella measurements. *Forensic Sci Int,* 95 (1), 39-45.

Irion DN, Schaffer AL, Famula TR, Eggleston ML, Hughes SS, Pedersen NC, 2003: Analysis of genetic variation in 28 dog breed populations with 100 microsatellite markers. *J Hered,* 94 (1), 81-87.

Jashari T, Kahvecioglu O, Duro S, Gundemir O, 2022: Morphometric analysis for the sex determination of the skull of the Deltari Ilir dog (Canis lupus familiaris) of Kosovo. *Anat Histol Embryol,* 51 (4), 443-451.

Klingenberg CP: MorphoJ, 2011: an integrated software package for geometric morphometrics. *Mol Ecol Resour,* 11 (2), 353-357.

Li A, Bennett D, Gibbs C, Carmichael S, Gibson N, Owen M, Denny HR, 2000: Radial carpal bone fractures in 15 dogs. *J Small Anim Pract,* 41 (2), 74-79.

Mastrangelo P, De Luca S, Alemán I, Botella MC, 2011b: Sex assessment from the carpals bones: discriminant function analysis in a 20th century Spanish sample. *Forensic Sci Int,* 206 (1-3), 216-e1.

Mastrangelo P, De Luca S, Sanchez-Mejorada G, 2011a: Sex assessment from carpals bones: discriminant function analysis in a contemporary Mexican sample. *Forensic Sci Int,* 209 (1-3), 196-e1.

Myo, N. Z, Po, SP, Soe, HY, Myint, HH, Khaing, KT, 2016: Comparison on Development of Ossification Centres of Radius and Ulna in Male and Female Dogs by Radiographically. *IJNRLS*, *3* (5), 18-29.

Olusa TA, Akbar Z, Murray CM, Davies HM, 2021: Morphometric analysis of the intercarpal ligaments of the equine proximal carpal bones during simulated flexion and extension of cadaver limbs. *Anat Histol Embryol,* 50 (1), 151-160.

Pedersen N, Liu H, Theilen G, Sacks B, 2013: The effects of dog breed development on genetic diversity and the relative influences of performance and conformation breeding. *J Anim Breed Genet,* 130 (3), 236-248.

Rainbow MJ, Crisco JJ, Moore DC, Wolfe SW, 2008: Gender differences in capitate kinematics are eliminated after accounting for variation in carpal size. *J Biomech Eng-t Asme,* 130 (4), 041003.

Riggs BL, Melton III LJ, Robb RA, Camp JJ, Atkinson EJ, Peterson JM, Rouleau PA, McCollough CH, Bouxsein ML, Khosla S, 2004: Population-based study of age and sex differences in bone volumetric density, size, geometry, and structure at different skeletal sites. *J Bone Miner Res,* 19 (12), 1945-1954.

Sarač-Hadžihalilović A, Ajanović Z, Hasanbegović I, Šljuka S, Rakanović-Todić M, Aganović I, Hadžiselimović R, 2022: Analysis of gender differences on pyriform aperture of human skulls using geometric morphometric method. *Folia Morphol,* 81 (3), 707-714.

Senol E, Gundemir O, Duro S, Szara T, Demiraslan Y, Karadag H, 2022.: A pilot study: Can calcaneus radiographic image be used to determine sex and breed in cats? *Vet Med Sci,* 8 (5), 1855-1861.

Szara T, Duro S, Gundemir O, Demircioglu İ, 2022: Sex determination in Japanese Quails (Coturnix japonica) using geometric morphometrics of the skull. *Animals,* 12(3), 302.

Vatne L, Dickson D, Tidholm A, Caivano D, Rishniw M, 2021: The effects of activity, body weight, sex and age on echocardiographic values in English setter dogs. *J Vet Cardiol,* 37, 26-41.

HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Immunohistochemical Alterations of Syndecan-1 in Sheep Liver with Cystic Echinococcosis

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Abstract: Cystic echinococcosis is formed by the larval forms of *Echinococcus granulosus*, causes health problems in humans and different species of animals worldwide. There is still limited information about the pathophysiological response to cystic echinococcosis in the liver. Syndecan-1 (Sdc1) is a cell surface proteoglycan of the liver. It has a crucial role in the pathophysiology of various liver diseases. This study aimed to investigate the immunohistochemical expression of Sdc1 in the liver with cystic echinococcosis in sheep. A total number of 51 liver tissue samples with cystic echinococcosis and ten healthy livers were examined. The tissue samples were stained with hematoxylin-eosin (HE) for histopathological examinations. Sdc1 was determined in the same liver tissues by immunohistochemistry. Infected liver tissues mainly showed severe inflammatory reactions, congestion, diffuse degeneration, and necrosis of hepatocytes around the cysts. The results indicated severe Sdc1-positive staining in hepatocytes around the cysts. Liver tissues of the control group showed relatively mild immunopositive reactions. This study determined that the Sdc1 significantly increased in the sheep livers with cystic echinococcosis infection.
Keywords: *Echinococcosis, Liver, Sheep, Syndecan-1.*

Kistik Ekinokokkozlu Koyun Karaciğerlerinde Sindekan-1'in İmmünohistokimyasal Değişiklikleri

Özet: Karaciğer kistik ekinokokkozisi *Echinococcus granulosus*'un larval formları tarafından oluşturulur. Dünya çapında insanlarda ve farklı hayvan türlerinde sağlık problemlerine neden olur. Kistik ekinokokkozisin patofizyolojisi hakkında hala sınırlı bilgi mevcuttur. Sindekan-1 (Sdc1) karaciğer hücre yüzey proteoglikanıdır. Farklı karaciğer hastalıklarının patofizyolojisinde önemli rolleri vardır. Bu çalışmada kistik ekinokokkozisli koyun karaciğerlerinde Sdc1 salınımındaki değişiklerin immünohistokimyasal olarak belirlenmesi amaçlanmıştır. Toplam 51 hidatid kistli karaciğer dokusu ve 10 sağlıklı karaciğer dokusu incelendi. Histopatolojik incelemeler amacıyla doku örnekleri hematoksilen-eosin (HE) ile boyandı. Aynı karaciğer dokularında Sdc1 immünohistokimyasal olarak belirlendi. Enfekte karaciğer dokularında başlıca şiddetli yangısal reaksiyon, konjesyon ve kist duvarları çevresindeki hepatositlerde yaygın dejenerasyon ve nekrozlar görüldü. Kist duvarları çevresindeki hepatositlerde şiddetli Sdc1 immünpozitif reaksiyon belirlendi. Kontrol grubuna ait karaciğer dokuları ise hafif immünopozitif reaksiyon gösterdi. Bu çalışmada ekinokokkozisli koyun karaciğer dokularında Sdc1 salınımın belirgin şekilde arttığı tespit edildi.
Anahtar Kelimeler: *Ekinokokkozis, Karaciğer, Koyun, Sindekan-1.*

Introduction

Cystic echinococcosis (hydatidosis) is a zoonotic parasitic disease caused by the larval forms of *Echinococcus granulosus.* It causes human and animal health problems in many regions of the world (McManus et al., 2003). In addition, it causes significant economic losses in meat, milk, fleece quality, and fertility (Budke, 2006; Widdicombe et al., 2022). Canids play a role in the transmission and spread of the infection. The infection has a high incidence in sheep breeding areas where dogs are common (Craig et al., 2007; Eckert et al., 2004). The cyst formation depends on the host's innate immunologic activity and the parasite's immunosuppression strategies (Cabrera et al., 1995; Rogan and Craig, 1997; Al Malki and Ahmed, 2022). It reacts to the parasite through free radicals that cause oxidative stress in the host. Free radicals cause oxidative damage to the liver when produced in excess (Derda et al., 2004; Kilic et al., 2010; Mert et al., 2019; Saleh, 2008). The main microscopic findings are degeneration and necrosis of hepatocytes, calcifications, and inflammation around the cyst in the infected liver (Heidarpour et al., 2012; Shoulah et al., 2023).

Sdc1 is the major cell surface heparan sulfate proteoglycan of the liver. It is predominantly expressed on the sinusoidal surface of hepatocytes. It provides cell-cell and cell-matrix connections as a regulator of cell behavior against various effectors (Afratis et al., 2017; Kim et al., 1994; Teng et al., 2012). They have many different functions, such as cellular adhesion, migration, and proliferation, with coreceptor in chemokines. The shedding of Sdc1 is triggered by tissue injury, inflammatory conditions, and mediators (Gopal et al., 2021; Teng et al., 2012). Sdc1 consists of a transmembrane, an intracellular, and an extracellular domain. In particular, the extracellular domain of this protein interacts with different ligands and promotes pathogenesis by facilitating pathogen attachment and invasion of cells (Nam et al. 2017; Palaiologou et al. 2014). Recent studies have shown that Sdc1 has a crucial role in the pathophysiology of various liver dysfunctions (Regos et al. 2020; Reszegi et al. 2022). Data have indicated that liver cells Sdc1 shedding in mice is activated during Schistosoma infection (Jacobs et al., 1999). Another study showed that in mice brains with infected toxoplasmosis, the brain cells overexpressed Sdc1 (Etewa et al., 2021). Beiting et al. (2006) observed that in muscle infection by Trichinella spiralis, the infected muscle cells produce Sdc1. A recent study demonstrated that Sdc1 overexpressed in sheep liver with natural fluke infection (Yumusak and Filikci., 2022). However, Bhanot and Nussenzweig (2002) concluded that Sdc1 does not play a role in experimental liver infection by Plasmodium sporozoites. On the other hand, Regos et al. (2020) found that Sdc1 was markedly expressed in cirrhotic liver. Matsumoto et al. (1997) observed a diffuse immunoreaction in hepatocellular carcinoma.

To our knowledge, no study has investigated before a possible relationship between Sdc1 and liver infected with *E. granulosus*. In this study, Sdc1 were investigated whether there is a significant difference between healthy and infected liver with cystic echinococcosis by immunohistochemistry.

Material and Method

Sample collection: A total of 51 sheep liver samples with hydatid cysts were used. And ten uninfected liver tissues taken from the slaughterhouse were examined as a control. The tissue samples were fixed in formaldehyde (pH 7.0) through immunohistochemical and histopathological studies. All stages of the study were carried out with the approval of the National Ethics Committee and under the supervision of the Local Ethics Commission (App. Number: 2023/002-01.17).

Gross and Histopathological Method: The affected livers were investigated for gross changes associated with the cysts. After routine pathologic tissue procedures, five micron- thick sections were stained with hematoxylin and eosin (HE). All slides were examined at x40-x100 objective magnifications under a light microscope (Olympus BX53-DIC; Tokyo, Japan).

Immunohistochemical Staining Method: Five-micrometer-thick sections were carried out on standard streptavidin-biotin peroxidase complex (ABC) technique protocol (Zymed, Histostain Plus Kit, California, USA) under 37°C and in humidity cabinets. The section was pre-treated with sodium citrate buffer (pH 6.0) using heat-mediated antigen retrieval for 20 min. Protein blocking was performed by incubating tissue sections with 5% normal bovine serum for 30 min before the primary antibody. The sections were incubated with Syndecan-1 (DL-101, sc-12765, Santa Cruz, USA), diluted at 1:50, overnight at 4°C. Biotinylated secondary antibody (TP-125-BN, Lab Vision, USA), was used to detect the primary antibody for 30 minutes, and diaminobenzidine (DAB, TA-125-HDS; Lab Vision Dako/Denmark) was used as the chromogen. The section was stained with hematoxylin for counterstain. The slides were semi-quantitatively scored according to the intensity of positive staining (-: no immunoreactivity; +: limited immunoreactivity; ++: strong immunoreactivity) (Jacobs et al., 1999).

Results

Gross Examination: The grossly multiple irregular spherical cysts were seen as superficial and fully deep embedded in the liver. Varying sizes of cysts were single or multiple. The cysts were generally soft and clear fluid-filled, ranging in size from 3 to 12 cm. Some cysts were firm, calcified, and hard to cut. The cut section of cysts was characterized by a white cyst membrane consisting of fibrous connective tissue.

Histopathological Findings: Liver tissues taken as control showed typical structures. The cyst wall had a thick, eosinophilic outer laminated membrane and a germinal epithelial layer (Figure 1a) in the infected liver. The cysts

were surrounded by the proliferating fibrous tissue, mononuclear cells, and eosinophils. In some chronic cysts were caseous or calcified. Diffuse degeneration and necrosis were observed in the hepatic lobules around the cyst structures. Hepatic parenchyma was congested and showed multiple small hemorrhages. Severe, multifocal inflammatory cell reactions were seen in nearby the cyst and portal areas, mostly of lymphocytes, macrophages, plasma cells, multinucleated giant cells, and a few eosinophils (Figure 1b). In addition, sinusoidal spaces and central veins were congested. Multifocally, proliferating fibrous tissue replaced adjacent hepatic parenchyma. The adjacent hepatocytes showed hypereosinophilic cytoplasm and necrosis. Vacuolar degenerative changes in hepatocytes were commonly seen in these areas. Pyknotic nuclei were detected in some hepatocytes. An increase in Kupffer cells was also observed in these areas. In addition, degeneration and hyperplasia were observed in the biliary epithelium, along with a few inflammatory cells.

Immunohistochemical Findings: Sdc1 mildly immunopositivity was detected in control liver samples (Figure 1c). In the livers of sheep with hydatid cysts, severe Sdc1- positive staining was seen, especially in the hepatocytes around the cyst wall (Figure 1d). It was determined that hepatocytes in areas of inflammation gave a severe Sdc1 immunoreaction. Hepatocytes in areas of degeneration and necrosis were found to have strongly positive staining. In addition, fibrous tissue cells around the cyst wall were mildly positive. However, immunopositivity was not detected in the cyst capsule and bile ducts.

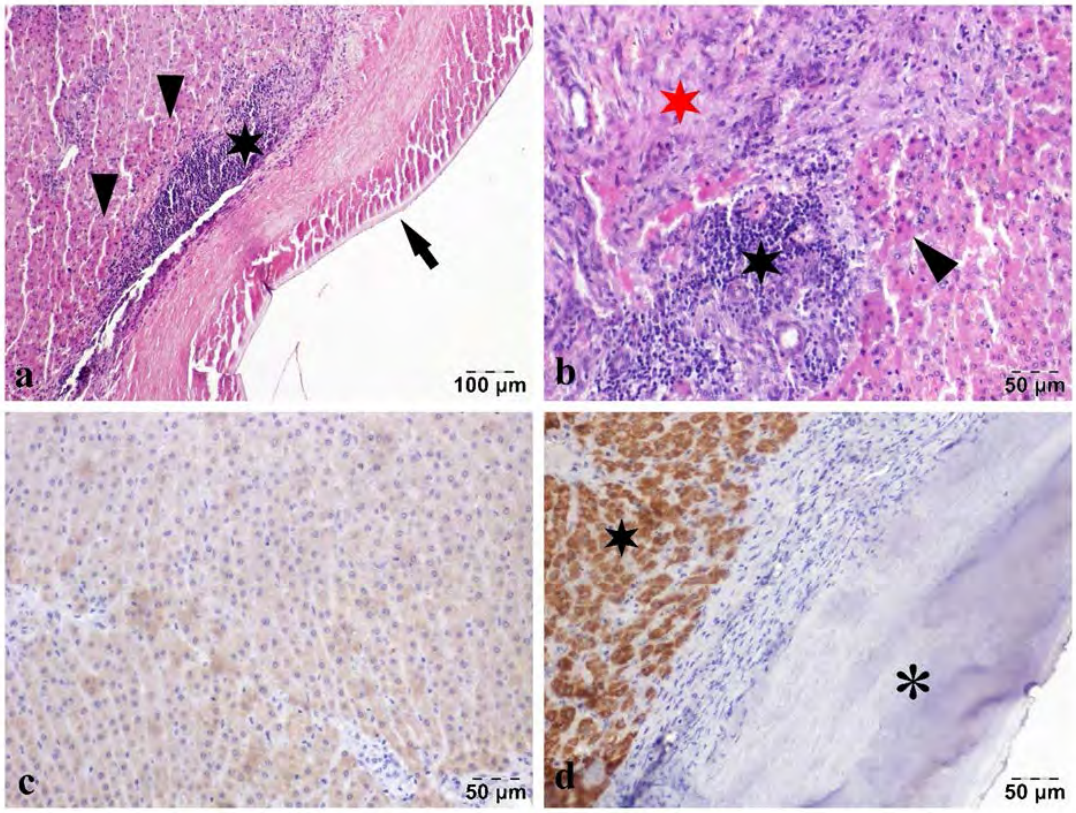

Figure 1. a) Severe inflammation around of cyst wall (star), degenerated hepatocytes (arrow heads) and cyst wall (arrow), HE. b) Severe inflammation (black star), fibrosis (red star) and degenerated hepatocytes (arrow head), HE. c) Mild Sdc1 immunpositive cells in control liver, DAB. d) Severe immunoreaction in infected liver cells (star) around of cyst wall (asterisk), DAB.

Discussion and Conclusion

In the present study, our main findings were *E. granulosus* caused severe hemorrhage, inflammatory cell infiltration, cellular degeneration, necrosis, fibrosis and, biliary hyperplasia, leading to significant liver damage as in previous studies. In addition, markedly increased levels of Sdc1 immunoexpression were determined in the infected liver, especially adjacent hepatocytes around the cysts. According to this limited study, overexpression of Sdc1 may play a role in *E. granulosus* induced liver damage in sheep.

Echinococcosis is an endemic zoonotic disease in several worldwide countries - larval forms *of E. granulosus* mainly cause illness of sheep. The larvae migrate through the circulatory system to several organs, especially the liver and lungs. The larvae in the liver destroyed the parenchyma, bile ducts, and vessels. Many studies documented these histopathological effects in sheep infected with *E. granulosus* (Al Malki and Ahmed, 2022; Budke, 2006; Widdicombe et al., 2022). The chronic granulomatous inflammatory process occurs as a characteristic finding of the disease. The cysts cause inflammatory reactions in the host, and severe congestion, hemorrhage, degeneration, and necrosis are

evident in the infected liver (Al Malki and Ahmed, 2022; Kebede et al., 2009; Serefettin et al., 2003). In some cases the cysts present an abscess or can be ruptured, producinf a pleural effusion or metastasis in the lungs and brain (Gerazounis et al. 2002). Ibrahim and Gameel (2014) have reported that severe fibrosis of the portal area in infected liver was detected. Singh et al. (2014) said that neighboring the cyst wall showed hepatocyte atrophy and cellular infiltration of lymphocytes, macrophages, and plasma cells around the fibrous capsule. Also, dilatation of sinusoids and central veins has been described. Khadidja et al. (2014) mentioned that extensive cirrhosis was observed in the infected liver in some cases. In the current study, disease's distinctive histopathological features were similar to the above-mentioned studies. Unlike this, we did not see severe cirrhosis, abscess or metastasis.

Sdc1 is a proteoglycan containing transmembrane heparan sulfate (HS). Sdc1 is released mainly from liver and other epithelial cells and plays a role in epitheliogenesis. Sdc1 studies in the literature still need to be improved, and the topic that has been mainly studied is the role of Sdc1 in the pathophysiology of liver diseases (Roskams et al., 1996; Zvibel et al., 2009). Recently, several authors have examined the anti-inflammatory effects of Sdc1 in clinical and experimental studies, while others have attempted to determine the potential antitumor effects of Sdc1 in liver carcinomas (Matsumoto et al., 1997; Nam et al., 2017; Regos et al., 2020; Roskams et al., 1996; Zvibel et al., 2009). Studies have shown that they are immunoexpressed in liver cirrhosis, infectious diseases such as hepatitis-c, hepatocellular carcinoma, and their metastases, as well as metastases of cholangiocellular carcinomas (Couchman, 2021; Reszegi et al., 2022). Roskams et al. (1996) determined that Sdc1 positivity is severe, especially in chronic cholestatic liver disease. They determined that Sdc1 immunoreactivity overexpressed the lateral and sinusoidal cell membrane of hepatocytes and the basolateral membrane of bile ductules. They suggested that Sdc1 may be critical in accompanying fibrogenesis and ductular reaction. In patients with HCV (chronic hepatitis C), especially in patients with cirrhosis and hepatitis, Sdc1 expression was significantly higher than in healthy individuals (Zvibel et al., 2009). Yılmaz et al. (2012) found that levels of Sdc1 significantly increased in serums of patients with nonalcoholic fatty liver disease. However, they found that this increase was not associated with histopathological liver damage, and similarly, they could not detect a correlation between immunohistochemical staining and serum Sdc1. Aquino et al. (2020) determined that in mice with Listeria monocytogenes, Sdc1 is expressed abundantly in the liver. Beiting et al. (2006) showed overexpressed Sdc1 in the Trichinella spiralis infection muscle cell cytoplasm. Yumusak and Filikci. (2022) demonstrated that Sdc1 was overexpressed in sheep liver infected by liver flukes. On the other side, Bhanot and Nussenzweig. (2022), declared that Sdc1 does not have a role in hepatocytes infected with Plasmodium yoelii.

In this study, we focused on the alteration of Sdc1 developed in cystic echinococcosis, and its alteration as the response to infected liver parenchyma. In our study the amount of Sdc1 varies in the liver infected with *E. granulosus* and healthy livers. The increased amount of Sdc1 were observed in all liver areas in infected livers. However, we observed strong immunoreactivity of Sdc1 in adjacent hepatocytes of cystic structures. On the contrary, mild immunoreactivity was seen fibrotic areas surrounding the cysts. Identifying targets for *E. granulosus* induced liver damage would be a basis for developing effective treatment strategies. The study with limited results concluded that Sdc1 release increased in the liver with cystic echinococcosis. These findings showed that Sdc1 proteins may have a role in that infection. However, more comprehensive molecular-related studies would clarify the pathogenesis of cystic echinococcosis in the liver.

Conflict of Interest

The authors stated that they did not have any real, potential, or perceived conflict of interest.

Ethical Approval

This study was performed with the permission of the Experimental Animals Local Ethics Committee in Harran University with (HRÜ-HADYEK) 10.05.2023 date and 01-17 approval number.

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Similarity Rate

We declare that the similarity rate of the article is 8% as stated in the report uploaded to the system.

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References

Afratis NA, Nikitovic D, Multhaupt HA, Theocharis AD, Couchman JR, Karamanos NK, 2017. Syndecans key regulators of cell signaling and biological functions, *FEBSJ*, 284, 27–41.

Al Malki J, Ahmed N, 2022: Epidemiological and histomorphic studies in sheep infected with hydatid cyst in Taif area. *Saudi J Biol Sci*, 29, 886-893.

Aquino RS, Hayashida A, Park PW, 2020: Host syndecan-1 promotes listeriosis by inhibiting intravascular neutrophil extracellular traps. *PLoS Pathogens*, 16 (5), e1008497.

Beiting DP, Park PW, Appleton JA, 2006: Synthesis of syndecan-1 by skeletal muscle cells is an early response to infection with Trichinella spiralis but is not essential for nurse cell development. *Infect Immun*, 74 (3), 1941-1943.

Bhanot P, Nussenzweig V, 2002: Plasmodium yoelii sporozoites infect Syndecan-1 deficient mice. *Mol Biochem Parasitol*, 2 (123), 143-144.

Budke CM, 2006: Global socioeconomic impact of cystic echinococcosis. *Emerg Infect Dis*, 12, 296–303.

Cabrera PA, Haran G, Benavidez V et al., 1995: Transmission dynamics of Echinococcus granulosus, Taenia hydatigena and Taenia ovis in sheep in Uruguay. *Int J Parasitol*, 25, 807–813.

Couchman JR, 2021: Syndecan-1 (CD138), carcinomas and EMT. *Int J Mol Sci*, 22, 4227.

Craig PS, McManus DP, Lightowlers MW et al., 2007: Prevention and control of cystic echinococcosis. *Lancet Infect Dis*, 7, 385-394.

Derda M, Wandurska-Nowak E, Hadas E, 2004: Changes in the level of antioxidants in the blood from mice infected with Trichinella spiralis. *Parasitol Res*, 93, 207–210.

Eckert J, Deplazes P, 2004: Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clin Microbiol Rev*, 17, 107-135.

Etewa S, Sarhan M, Moawad H, Mohammad S, Samir M, Kandil A, Mostafa E, 2021: Behavior and neuropsychiatric changes in experimental chronic toxoplasmosis: Histopathological and immunohistochemical studies. *Parasitologists United Journal*, 14 (2), 183-192.

Gerazounis M, Athanassiadi K, Metaxas E, Athanassiou M, Kalantzi N, 2002: Bronchobiliary fistulae due to echinococcosis. *Eur J Cardiothorac Surg*, 22 (2), 306-308.

Gopal S, Arokiasamy S, Pataki C, Whiteford JR, Couchman JR, 2021: Syndecan receptors: pericellular regulators in development and inflammatory disease. *Open Biol*, 11, 200377.

Heidarpour M, Mohri M, Borji H, Moghdass E, 2012: Oxidative stress and trace elements in camel (Camelus dromedarius) with liver cystic echinococcosis. *Vet Parasitol*, 187, 459–463.

Ibrahim SEA, Gameel AA, 2014: Pathological, histochemical and Immunohistochemical studies of lungs and livers of cattle and sheep infected with hydatid disease. In: Proceedings of 5th annual conference-agricultural and veterinary research–February 2014, Khartoum, Sudan, 2, 1–17.

Jacobs W, Bogers J, Van Marck E, 1999: Distinct B-cell populations are present in hepatic and intestinal Schistosoma mansoni granulomas. *Acta Gastroenterol Belg*, 62 (2):178-81.

Kebede N, Mitiku A, Tilabun G, 2009: Hydatidosis of slaughtered animals in Bahir Dar abattoir, northwestern Ethiopia. *Trop Anim Health Prod*, 41, 43-50.

Khadidja H, Achour Y, Houcin B, Vasile C, 2014: Histological Appearance of Echinococcus Granulosus in the Camel Species in Algeria. *Bulletin UASVM Vet Med*, 71, 79–84

Kılıç E, Yazar S, Başkol G, Artiş T, Ersayit D, 2010: Antioxidant and nitric oxide status in patients diagnosed with Echinococcus granulosus. *Afr J Microbiol Res*, 4, 2439- 2443.

Kim CW, Goldberger OA, Gallo RL, Bernfield M, 1994: Members of the syndecan family of heparan sulfate proteoglycans are expressed in distinct cell-, tissue-, and development-specific patterns, *Mol Biol Cell*, 5, 797–805.

Matsumoto A, Ono M, Fujimoto Y, Gallo RL, Bernfield M, Kohgo Y, 1997: Reduced expression of syndecan-1 in human hepatocellular carcinoma with high metastatic potential. *Int J Cancer*, 74, 482-491.

McManus DP, Zhang W, Li J, Bartley PB, 2003: Echinococcosis. *Lancet*, 362, 1295–1304.

Mert H, Yaşar S, Mert N, 2019: Investigation of TAS and TOS levels in sheep with hydatid cyst. *EurasianJHS*, 2 (4), 164-168.

Nam EJ, Hayashida K, Aquino RS, Couchman JR, Kozar RA, Liu J, Park PW, 2017: Syndecan-1 limits the progression of liver injury and promotes liver repair in acetaminophen-induced liver injury in mice. *Hepatology*, 66, 1601-1615.

Palaiologou M, Delladetsima I, Tiniakos D, 2014: CD138 (syndecan-1) expression in health and disease, *Histol Histopathol*, 29, 177-189.

Regos E, Karászi K, Reszegi A, Kiss A, Schaff Z, Baghy K, Kovalszky I, 2020: Syndecan-1 in liver diseases. *Pathol Oncol Res*, 26, 813-819.

Reszegi A, Tátrai P, Regős E, Kovalszky I, Baghy K, 2022: Syndecan-1 in liver pathophysiology. *Am J Physiol Cell Physiol*, 323, 289-294.

Rogan MT, Craig PS, 1997: Immunology of Echinococcus granulosus infections. *Acta Tropica*, 67, 7-17.

Roskams T, Rosenbaum J, De Vos R, David G, Desmet V, 1996: Heparan sulfate proteoglycan expression in chronic cholestatic human liver diseases. *Hepatology*, 24, 524-532.

Saleh MA, 2008: Circulating oxidative stress status in desert sheep naturally infected with Fasciola hepatica. *Vet Parasitol*, 154, 262–269.

Serefettin M, Merih G, Tulay C, Astarcioglu H, 2003: The Pathology of Echinococcosis and the Current Echinococcosis Problem in Western Turkey (A Report of Pathologic Features in 80 Cases). *Turk J Med Sci*, 33, 369-374.

Shoulah SA, Gaballa MM, Marawan MA, Saqr SA, Abdelhady A, Alzahrani HA, Wakid M, Al-Jabr O, Selim A, 2023: Pathological Findings and Oxidative Stress Status Associated with Hydatidosis in Dromedary Camels. *Vet Sci*, 10, 74.

Singh BB, Sharma R, Sharma JK, Mahajan V, Gill JPS, 2014: Histopathological changes associated with E. Granulosus echinococcosis in food producing animals in Punjab (India). *J Parasit Dis*, 40, 1–3

Teng YHF, Aquino RS, Park PW, 2012: Molecular functions of syndecan-1 in disease. *Matrix Biol*, 31, 3-16.

Widdicombe J, Basáñez MG, Entezami M, Jackson D, Larrieu E, Prada JM, 2022: The economic evaluation of Cystic echinococcosis control strategies focused on zoonotic hosts: A scoping review. *PLoS Negl Trop Dis*, 16 (7), e0010568.

Yilmaz Y, Eren F, Colak Y, Senates E, Celikel CA, Imeryuz N, 2012: "Hepatic expression and serum levels of syndecan 1 (CD138) in patients with nonalcoholic fatty liver disease". *Scand J Gastroenterol*, 47 (12), 1488-1493.

Yumusak N, Filikci K, 2022: Immunohistochemical Evaluation of Syndecan-1 Expression in the Liver of Small Ruminants with Natural Liver Fluke Infection. *Harran Univ Vet Fak Derg*, 11, 73-77.

Zvibel I, Halfon P, Fishman S, Penaranda G, Leshno M, Or AB, Halpern Z, Oren R, 2009: Syndecan 1 (CD138) serum levels: a novel biomarker in predicting liver fibrosis stage in patients with hepatitis C. *Liver Int*, 29, 208-212.

HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Surgical Time for Laparoscopic Ovariectomy in Adult and Prepubertal Dogs

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Abstract: Two-portal laparoscopic ovariectomy (LOVE) was performed in adult (n=23) and prepubertal dogs (n=23) to compare surgical time and to evaluate the effect of age, body weight (BW), body condition score (BCS), ovarian pedicle fat score (OPFS) and intraoperative surgical complications on surgical time. Three classes of BCS were considered: BCS 1-2=lean; BCS 3=ideal; BCS 4-5=overweight. The same surgeon performed all surgeries. OPF was scored and was considered negative or positive. Intraoperative complications were recorded. Total surgical time was recorded from the first skin incision to the last portal closure suture placement. The surgical procedure was divided into seven stages, and each stage's time was recorded. Data are reported as mean±SEM. Differences in the duration of defined surgical stages between groups were evaluated by age, BW, OPFS and intraoperative complications. One-way analysis of variance was used to assess the effect of BCS. No major complications were observed. Five and four minor complications were observed in adult and prepubertal dogs, respectively. Total surgical time was not different ($p>0.05$) between adult (20.63±0.60 min) and prepubertal (21.48±1.75 min) dogs. BCS, BW, and OPF did not affect total surgical time ($p>0.05$). Intraoperative complications have prolonged surgical procedure times (25.20±2.33 min vs 20.04±0.93 min). The time from the first skin incision to the insertion of the first trocar was shorter ($p<0.05$) in prepubertal dogs (2.38±0.12 min) compared to adult animals (2.71±0.10 min). The time needed for grasping of the right ovary was longer ($p<0.05$) in prepubertal dogs (3.02±0.31 *vs* 2.32±0.16 min) and in animals without OPF (2.94±0.24 *vs* 2.11±1.89 min). In conclusion, LOVE is safe in adult and prepubertal dogs with similar surgical time and complication rates.

Keywords: *Dog, Laparoscopy, Ovariectomy, Prepubertal, Surgical time.*

Yetişkin ve Prepubertal Köpeklerde Laparoskopik Overiektomi Cerrahi Süresi

Özet: Cerrahi sürenin karşılaştırılması ve yaş, vücut ağırlığı (VA), vücut kondisyon skoru (VKS), ovaryan pedikül yağ skoru (OPYS) ve intraoperatif cerrahi komplikasyonların cerrahi süre üzerine etkilerinin araştırılması amacıyla yetişkin (n=23) ve prepubertal köpeklerde (n=23) iki-portal laparoskopik ovariektomi (LOVE) yapılmıştır. Üç VKS sınıfı dikkate alınmıştır: VKS 1-2=zayıf; VKS 3=ideal; VKS 4-5=fazla kilolu. Tüm ameliyatlar aynı cerrah tarafından yapılmıştır. OPY skorlanmış ve negatif veya pozitif olarak kabul edilmiştir. İntraoperatif komplikasyonlar kaydedilmiştir. Toplam cerrahi süre, ilk deri ensizyonundan son portal kapatma sütürünün yerleştirilmesine kadar geçen süre olarak kaydedilmiştir. Cerrahi işlem 7 farklı aşamaya ayrılmış ve her aşamanın süresi kaydedilmiştir. Veriler ortalama±SEM olarak sunulmuştur. Gruplar arasında tanımlanmış cerrahi sürelere yaş, VA, OPYS ve intraoperatif komplikasyonların etkisi ile değerlendirilmiştir. VKS etkisinin değerlendirilmesi için tek yönlü varyans analizi kullanılmıştır. Majör komplikasyon ile karşılaşılmamıştır. Erişkin ve prepubertal köpeklerde sırasıyla 5 ve 4 minör komplikasyon gözlenmiştir. Erişkin (20,63±0,60 dk) ve prepubertal (21,48±1,75 dk) köpekler arasında toplam cerrahi süre farklılık göstermemiştir ($p>0,05$). VKS, VA ve OPY toplam cerrahi süresini etkilememiştir ($p>0,05$). İntraoperatif komplikasyonlar cerrahi süreyi uzatmıştır (25,20±2,33 dk; 20,04±0,93 dk). İlk deri ensizyonundan ilk trokarın yerleştirilmesine kadar geçen süre prepubertal köpeklerde (2,38±0,12 dk) erişkin hayvanlara (2,71±0,10 dk) kıyasla daha kısa olmuştur ($p<0,05$). Sağ ovaryumu kavramak için gereken süre prepubertal köpeklerde (3,02±0,31 dk; 2,32±0,16 dk) ve OPY negatif hayvanlarda (2,94±0,24; 2,11±1,89 dk) daha uzun olmuştur ($p<0,05$). Sonuç olarak LOVE, benzer cerrahi süre ve komplikasyon oranları ile yetişkin ve prepubertal köpeklerde güvenli bulunmuştur.

Anahtar Kelimeler: *Cerrahi süre, Köpek, Laparoskopi, Ovariektomi, Prepubertal.*

Introduction

Elective sterilization in dogs is the most commonly performed surgical procedure in veterinary practice (DeTora and McCarthy, 2011; Tez et al., 2019). Many techniques for surgical sterilization in dogs have been described, each offering advantages and disadvantages to both the patient and surgeon. The surgeon has to choose the least invasive, fastest, and safest procedure. Technically, ovariectomy (OVE) can be performed with a smaller incision and less tissue trauma due to the absence of corpus uteri and suspended ligaments' disturbance. Thus, the surgical time for ovariectomy is shorter than for ovariohysterectomy (Tallant et al., 2016; Van Goethem et al., 2006).

Minimally invasive surgery has long been suggested as an alternative to traditional ovariohysterectomy or ovariectomy (Manassero and Viateau, 2018). With the development of minimally invasive surgical techniques, laparoscopic ovariectomy has gained popularity in veterinary medicine (Culp et al., 2009). In particular, increasing public awareness has led pet owners to adopt a less invasive approach to surgical interventions for their pets. In addition, the number of veterinarians who offer laparoscopic methods to their patients is increasing rapidly (Buote, 2022; Hsueh et al., 2018). Today, laparoscopic surgery in dogs is mainly applied for elective sterilization (Findji, 2014; Tez and Kanca, 2018).

The advantages of laparoscopic ovariectomy (LOVE) are better visibility, smaller incisions, reduced ovarian remnant syndrome risk, decreased surgical trauma, shortened healing time, atraumatic cauterization, and less surgical pain and stress. (Marvel, 2022). These advantages are promising when surgical time, hospitalization duration, complication rate, and cost-intensive sterilization programs are important. However, the cost of tools and equipment, potential intraoperative complications, and the need for specific training limit their widespread use (Tez and Kanca, 2018).

The term prepubertal is generally used for dogs from 6 to 14 weeks, although it defines the period before the onset of sexual activity (Stubbs and Bloomberg, 1995). Prepubertal sterilization is routinely performed in animal rehabilitation centers, especially in the United States and the United Kingdom, because it is considered the easiest and most effective population control method (Moxon et al., 2023). Many veterinarians and institutions advocate prepubertal or pre-adoption spaying to prevent the rapid population increase, maintenance and hosting cost reduction, and the risk of mammary neoplasia in dogs (Howe et al., 2001; Valdez, 2022). Even so, there is an ongoing dilemma about shortening surgical time through better visibility or small size of reproductive tissues and high tissue fragility causing harsh procedures (Howe, 2006; Stubbs and Bloomberg, 1995).

Laparoscopic OVE has not been extensively studied in prepubertal dogs, and controlled studies comparing the duration of surgical sterilization of prepubertal and adult female dogs are warranted. Therefore, we aimed to investigate the effects of body weight, body condition score, ovarian pedicle fat status, and intraoperative complications on laparoscopic ovariectomy surgical time in both prepubertal and adult dogs.

Material and Methods

The study procedure was approved by the Ankara University Local Ethics Committee of Animal Experiments (No: 2016-5-53). We used 46 (23 adult and 23 prepubertal) female dogs weighing 8-44 kg. Anamnesis, physical examination, complete blood count, abdominal ultrasonography, and vaginal cytology were used to determine the suitability of all dogs for surgical sterilization. Vaginal smear samples were taken from adult dogs to determine the sexual cycle stage. The sexual cycle stage was determined in smear samples stained with the Papanicolaou method (Feldman and Nelson, 2004). Adult dogs out of the follicular phase and prepubertal were included.

All surgical procedures were performed under the same anesthesia and analgesia protocol. A single dose of cefazolin (20 mg/kg, IM) was applied 30 min before surgery. All dogs were premedicated with medetomidine hydrochloride (0.04 mg/kg). For the induction of anesthesia, propofol (2-3 mg/kg) was administered intravenously (IV). After intubation, general anesthesia was maintained with isoflurane (1%, Isoflurane) in 50% oxygen.

The dogs were placed in the dorsal recumbent position on a special operating table that could tilt to the right and left. The ventral abdomen was shaved from the xiphoidal region to the pubis, and the urinary bladder was examined by ultrasonography. In cases where the bladder is full enough to affect the operation's success, the urine is emptied by abdominal compression or catheterization. After cleaning the operation area with soapy water, asepsis was provided with povidone-iodine.

One surgeon (G.T.) performed all surgeries. All dogs received lactated ringer solution (5 ml/kg/hr, IV) and were monitored during surgery. A two-portal technique and an electrothermal bipolar vessel sealing device (LigaSure™) were used. A 7 mm skin incision was made 2 cm caudal to the umbilicus. The first 5 mm trocar (VersaOne™ Bladed Trocar, Covidien, Turkey) was inserted using Hasson's technique. Pneumoperitoneum was achieved with pressure of 10-12 mmHg. The second trocar (11-12 mm, VersaOne™ Bladed Trocar, Covidien, Turkey) was inserted through a skin incision halfway between the first incision and the pubis. A 5 mm diameter, 30° angle of a laparoscope (30 cm, Eickemeyer®, Germany) was introduced into the cranial cannula, and a grasping forceps (5 mm, 36 cm, Eickemeyer®, Germany) into the caudal cannula. The left ovary was grasped and pulled to the abdominal wall with the dog in the right lateral recumbency. A transabdominal suspension suture was used to fix the ovary. The associated ligaments were sealed and transected using a laparoscopic vessel sealing device (LigaSure™, 5 mm, 37 cm, Blunt Tip, Covidien, Turkey). The ovary was removed from the abdomen through the portal. The dog was positioned in left lateral recumbency, and the procedure was repeated for the right

ovary. Pneumoperitoneum was released, and abdominal portals were closed in 2 layers.

Body weight (BW), body condition score (BCS), ovarian pedicle fat score (OPFS), and intraoperative complications were recorded. Body condition was scored using a 1-5 point BCS system (Impellizeri et al., 2000). The dogs were grouped as lean (BCS 1-2), ideal (BCS 3), and overweight (BCS 4-5). Ovarian pedicle fat was scored during operations (Van Nimwegen and Kirpensteijn, 2007) from 0 to 3, and OPF status was considered negative (OPFS= 0-1) or positive (OPFS=2-3).

Intraoperative complications were recorded as minor or major. Bleeding from the spleen, ovarian bursa, pedicle or other iatrogenic abdominal trauma were characterized as minor intraoperative complications. Major complications were evaluated as requiring intraoperative conversion to laparotomy or postoperative revision.

In order to compare the surgical times between the groups, the total time and the times for all surgical stages were recorded separately. Total surgical time was recorded from the first skin incision to the last portal closure. The surgical procedure was divided into seven stages, and the time for each step was recorded: Skin incision until first trocar insertion (1); insertion of second trocar (2); grasping of left ovary (3); removal of left ovary (4); grasping of right ovary (5); removal of right ovary (6); abdominal portal closure (7).

The Student's t-test was used to examine the effects of age (prepubertal and adult) and OPF on surgical time and to compare surgical times. One-way analysis of variance (ANOVA) was used to examine the differences in surgical time in terms of study groups and BCS groups. Tukey's test was applied as a post hoc test for groups that were found to be significant. Descriptive statistics on surgical time and demographic findings are shown as "Arithmetic Mean±Standard Error". Pearson correlation analysis was used to determine the strength and direction of the relationship between surgery time and body weight. SPSS 14.01 (License No: 9869264) package program was used to analyze the data. The $p<0.05$ criterion was taken into account in all statistical decisions.

Results

The mean age of the animals used in the study was 6.35±0.04 months (5-8 months) in prepubertal dogs and 21.17±0.36 months (10-48 months) in adult dogs. In prepubertal and adult dogs the mean body weight was 15.39±0.18 kg (9-27 kg) and 23.35±0.36 kg (13-42 kg). Most prepubertal dogs (n=10) had a BCS of 3. Ten prepubertal dogs were classified as lean. A BCS of 5 was not observed in prepubertal dogs. However, three dogs were classified as overweight. Three adult dogs were lean. The body condition of 14 adult dogs was ideal, and six dogs were overweight. Only two prepubertal dogs have ovarian pedicle fat. In contrast, 13 adult dogs were classified as OPF-positive. The mean age, BW, BCS, and OPFS of dogs in study groups are presented in Table 1.

Table 1. The mean age, body weight, body condition score and ovarian pedicle fat score in prepubertal and adult dogs.

Group	n	Age (month)	Body Weight (kg)	Body Condition Score (1-5)	Ovarian Pedicle Fat Score (0-3)
pLOVE[1]	**23**	6.35±0.04	15.39±0.18	2.52±0.04	0.44±0.03
aLOVE[2]	**23**	21.17±0.36	23.35±0.36	3.17±0.36	1.74±0.04

[1]Prepubertal laparoscopic ovariectomy.
[2]Adult laparoscopic ovariectomy.

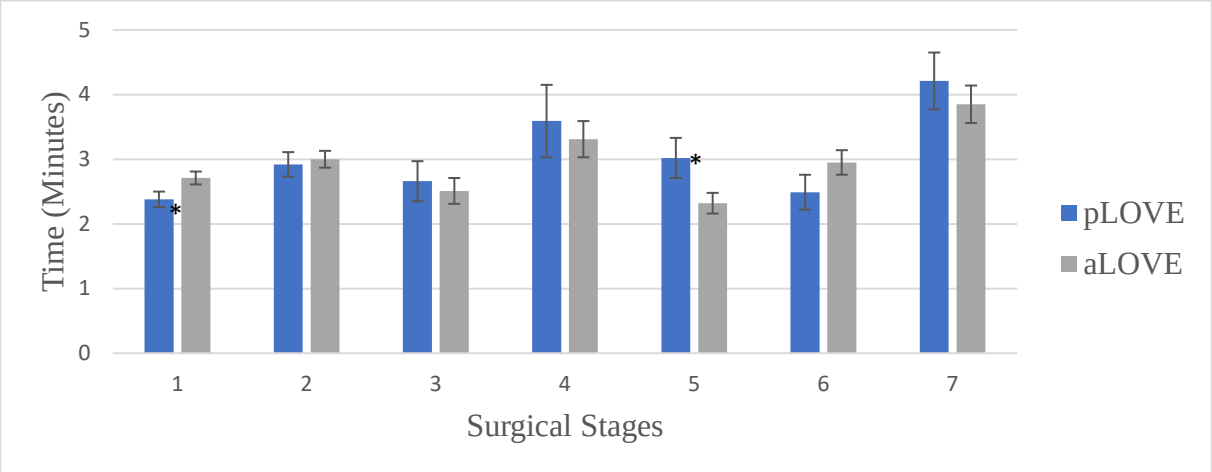

Figure 1. Surgical stage times during laparoscopic ovariectomy in prepubertal and adult dogs.
1st stage: skin incision until first trocar insertion; 2nd stage: insertion of second trocar; 3rd stage: grasping of left ovary; 4th stage: removal of left ovary; 5th stage: grasping of right ovary; 6th stage: removal of right ovary; 7th stage: abdominal portal closure. *p<0.05: represents statistically significant difference among groups in the same surgical stage. pLOVE: prepubertal laparoscopic ovariectomy; aLOVE: adult laparoscopic ovariectomy.

No difference was observed in total surgical time between prepubertal (pLOVE: 21.48±1.75 min) and adult (aLOVE: 20.63±0.60 min) dogs (p>0.05). Closure of the portals was the most time-consuming stage during LOVE (pLOVE: 4.21±0.44 min; aLOVE: 3.85±0.29 min, p>0.05). In pLOVE, the time needed for the first trocar insertion was shorter (p=0.044); in contrast, the time required for grasping the right ovary was longer than aLOVE (p<0.05). The time needed for insertion of the second trocar (pLOVE: 2.92±0.19 min; aLOVE: 3.00±0.13 min), grasping of left ovary (pLOVE: 2.66±0.31 min; aLOVE: 2.51±0.20 min), removal of left ovary (pLOVE: 3.59±0.56 min; aLOVE: 3.31±0.28 min) and removal of right ovary (pLOVE: 2.49±0.27 min; aLOVE: 2.95±0.19 min) did not differ among the groups (p>0.05). A comparison of surgical stage times between prepubertal and adult LOVE is given in Figure 1.

No relationship was observed between body weight and total surgical time (p=0.37). Laparoscopic OVE total surgical time and surgical stage times were not affected by BCS (p>0.05). Total surgical time in lean, ideal, and overweight dogs were 18.45±1.27 min, 22.82±1.52 min, and 20.10±0.83 min, respectively. Total surgical times of LOVE were not affected by OPF (p>0.05). Total surgical time was 20.04±0.57 min in OPF (+) dogs and 21.54±1.33 min in OPF (-) dogs. However, the grasping of the right ovary during LOVE was shorter (p=0.029) in OPF-positive dogs (2.11±1.89 min) compared to OPF-negative dogs (2.94±0.24).

A total of 9 minor intraoperative complications were identified (19.6%), and no major intraoperative complications were observed during LOVE. All intraoperative complications were minor haemorrhagia due to tissue damage originating from intestines (n=1; 2.2%), urinary vesicle (n=1; 2.2%), spleen (n=3; 6.6%) and ovarian pedicle (n=4; 8.7%) haemorrhagia. Only for ovarian pedicle haemorrhagia Ligasure™ (Covidien, Medtronic, Türkiye) was used: none others needed intervention.

Total surgical time was longer in the presence of intraoperative complications (20.04±0.93 min vs. 25.20±2.33 min; p=0.024). Time for the second trocar insertion was longer in LOVE with intraoperative complications (3.54±0.22 min) when compared to LOVE (2.82±0.12 min) without complications (p=0.009). Intraoperative complications did not affect other surgical stage times of LOVE (p>0.05).

Discussion and Conclusion

Laparoscopic OVE surgery time is affected by several factors, such as the number of ports, pneumoperitoneum induction method, imaging system, hemostasis or resection method, and operator experience. Consequently, different studies reported varying surgical times for LOVE (Freeman et al., 2010; Van Nimwegen and Kirpensteijn, 2007). In a study investigating the effects of the number of ports, total surgical times for single and double portal LOVE were similar. However, the duration of surgery extended in three portal techniques (Case et al., 2011). The use of a single port in laparoscopic OVE provides an aesthetic advantage (Manassero et al., 2012); however, it is disadvantageous compared to the double portal technique in terms of maneuverability and ease of access (Case et al., 2011; Dupre et al., 2009). Therefore, the most commonly used method in dogs is two-portal LOVE.

As an alternative to mechanical methods (ligature, clip, staple) for hemostasis during LOVE in dogs, monopolar and bipolar electrocoagulation (Van Goethem et al., 2003), laser technology (Van Nimwegen and Kirpensteijn, 2007; Van Nimwegen et al., 2005), ultrasonic devices (Hancock et al., 2005), and electrothermal bipolar vessel sealing instruments (Culp et al., 2009; Dupre et al., 2009; Mayhew and Brown, 2007) have been reported. The current hemostasis method recommended for LOVE in dogs is electrothermal bipolar vessel sealing (Culp et al., 2009; Öhlund et al., 2011). In the present study, a two-portal technique and an electrothermal bipolar vessel sealing device (Ligasure™, Covidien, Medtronic, Turkey) were chosen to minimize the possible negative effects on surgical times. As a result, LOVE surgical times were shorter than previously reported studies using different port numbers and instrumentation (Van Goethem et al., 2003; Van Nimwegen and Kirpensteijn, 2007; Van Nimwegen et al., 2005). The LigaSure™ system sends a warning signal when coagulation is complete, and it shortens the surgical time by allowing the operator to cut manually and remove the ovaries without changing instruments in between. In addition, Ligasure™ causes less damage to the surrounding tissues by creating a heat dissipation of up to 1.5 mm, reducing the rate of intraoperative complications (Culp et al., 2009; Öhlund et al., 2011). Similar LOVE total surgery times have been reported in studies using the same technique. Case et al. (2011) reported that LOVE was completed in 19.1±6.3 min. In a Dupre et al. (2009) study, the total surgical time was 18.2±4.4 min.

It has been suggested that the surgical time will be shortened due to better visibility in prepubertal sterilization (Stubbs and Bloomberg, 1995). On the other hand, small reproductive tissue sizes and high tissue fragility can adversely affect the total surgical time. Although total surgical times were not different in the current study, inserting of the first trocar was shorter in prepubertal dogs. Conversely, the time needed for grasping the right ovary was longer. The incision time of the thin abdominal layers is less in prepubertal dogs. The current study used the open technique to insert the first trocar. In this technique, abdominal muscle and skin incisions can be performed more quickly in prepubertal dogs. In addition, the presence of subcutaneous adipose tissue in adult dogs may cause prolongation of the surgical stage.

It has been reported that it is more difficult to reach the ovaries in dogs under one year of age during LOVE, and the sexual cycle stage plays an essential role in detecting the ovaries (Van Nimwegen and Kirpensteijn, 2007). The faster detection of the right ovary, located more cranially in adult dogs, is attributed to the larger uterine and ovarian tissue and reproductive hormone exposure. In the current study, vaginal cytological examination was performed, and dogs in the follicular phase of the sexual cycle were omitted. Therefore, the authors believe there was no effect of the sexual cycle stage on surgical time. The authors further believe that controlled ligation of the thin and sensitive

ovarian pedicle in prepubertal dogs requires more time to remove the more cranially located right ovary.

Body weight did not affect the total surgical time in any of the study groups. In two studies evaluating the effects of body weight on the total surgical time of LOVE, no significant effect was observed (Öhlund et al., 2011; TapiaAraya et al., 2015). On the other hand, Dupre et al. (2009) reported that body weight affects the total surgical time, and surgery is prolonged in dogs with a body weight of <7 kg and >33 kilograms. Body weight is a criterion for identifying dogs suitable for LOVE. Dogs weighing >8-10 kg were used in almost all studies (Hancock et al., 2005; Mayhew and Brown, 2007; Van Goethem et al., 2003; Van Nimwegen and Kirpensteijn, 2007). In addition, a prerequisite of >8 kg is required for dogs to be treated with LOVE at the Royal Veterinary College. In dogs with a body weight of >33 kilograms, it was reported that the duration of surgery was prolonged due to the inadequacy of hand instruments with a working length of 37 cm, and the use of longer laparoscopic instruments has been suggested (Dupre et al., 2009). In the current study, 37 cm long hand instruments were used successfully.

In the current study, BCS did not affect total surgical time and surgical stage times. Two studies using techniques similar to the present study reported that BCS did not affect the total surgical time of LOVE (Öhlund et al., 2011; Tapia-Araya et al., 2015). It has been suggested that LOVE may be an alternative to traditional OVE by visualizing the ovaries in obese dogs with high abdominal fat and requiring wide incisions (Van Goethem et al., 2003). Unaffected LOVE times in the current study reveal the advantage of LOVE in the surgical sterilization of obese dogs and support the abovementioned suggestion.

Ovarian pedicle fat is considered the most important factor affecting the duration of surgery (Harris et al., 2013). The presence of OPF increases the total surgical time of canine LOVE using laser and bipolar electrocoagulation methods (Van Nimwegen and Kirpensteijn, 2007), and the total duration of surgery and the duration of the surgical stage was longer in small-sized dogs with high OPFS (Granados et al., 2017). However, the total surgical time was not affected by OPY in two studies with Ligasure™ (Öhlund et al., 2011; Tapia-Araya et al., 2015). In the current study, LOVE total surgery time was unaffected by OPF, and the time needed to identify the right ovary was shorter in dogs with OPF. The lacking effect of OPF on the total surgical time of LOVE has been associated with the ease in hemostasis of ovaries in the current study. The shorter time needed to identify the right ovary in dogs with OPF may be attributed to periovarian fat resulting in better visibility.

No major complications were observed during LOVE in the current study. This result is consistent with the fact that no major complications requiring conversion to open surgery were reported in the last 20 years (Case et al., 2011; Van Goethem et al., 2003; Van Nimwegen and Kirpensteijn, 2007). No major intraoperative complications were reported in a large retrospective study of 618 LOVE, (Pope and Knowles, 2014). More strikingly, no major complications were observed in 161 LOVE performed by Colorado State University fourth-year students (Nylund et al., 2017). As a result, LOVE is a very safe method for intraoperative complications for both prepubertal and adult dogs. In addition, the availability of open surgery when necessary is an essential advantage for LOVE.

Intraoperative complications in the study resulted in longer total surgery times. In terms of surgical stage times, only the time taken for insertion of the second trocar was affected by intraoperative complications. Complications during laparoscopic sterilization are directly related to the suitability of the equipment used and the operator's experience. Intraoperative complications during LOVE using Ligasure™ usually occur due to minor hemorrhage, do not require intervention, and hemostasis is achieved spontaneously (Culp et al., 2009). Furthermore, it is possible to intervene in hemorrhages.

It has been concluded that LOVE is a safe method for surgical sterilization of prepubertal and adult dogs. Total surgery time is similar in prepubertal and adult dogs and is not affected by body weight and body condition or OPF. Furthermore, grasping of the right ovary during LOVE is shorter in dogs with OPF. Minor intraoperative complications prolong the total surgical times of LOVE by increasing the time needed for the second trocar insertion.

Conflict of Interest

The authors stated that they did not have any real, potential or perceived conflict of interest.

Ethical Approval

This study was approved by the Ankara University Animal Experiments Local Ethics Committee (24.02.2016, 2016-5-53 Number Ethics Committee Decision). In addition, the authors declared that Research and Publication Ethical rules were followed.

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Similarity Rate

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Author Contributions

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Design: GT, HK
Control/Supervision: GT, HK
Data Collection and / or Processing: GT, HK
Analysis and / or Interpretation: GT, HK
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Writing the Article: GT, HK, SE
Critical Review: SE

References

Buote NJ, 2022: Updates in laparoscopy, *Vet Clin N Am Small Anim Pract,* 52 (2), 513-529.

Case JB, Marvel SJ, Boscan P, Monnet EL, 2011: Surgical time and severity of postoperative pain in dogs undergoing laparoscopic ovariectomy with one, two, or three instrument cannulas. *J Am Vet Med Assoc,* 239 (2), 203-208.

Culp WTN, Mayhew PD, Brown DC, 2009: The effect of laparoscopic versus open ovariectomy on postsurgical activity in small dogs. *Vet Surg,* 38, 811-817.

Detora M, McCarthy RJ, 2011: Ovariohysterectomy versus ovariectomy for elective sterilization of female dogs and cats: Is removal of the uterus necessary? *J Am Vet Med Assoc,* 239 (11), 1409-1412.

Dupre G, Fiorbianco V, Skalicky M, Gultiken N, AY SS, Fındık M, 2009: Laparoscopic ovariectomy in dogs: Comparison between single portal and two-portal access. *Vet Surg,* 38, 818-824.

Feldman EC, Nelson RW, 2004: Ovarian cycle and vaginal cytology. In: Canine and Feline Endocrinology and Reproduction (4th Ed.), Saunders, Elsevier Health Sciences, Missouri, p.: 770-773.

Findji L, 2014: Ovariohysterectomy vs ovariectomy. *Clinician Brief,* 3, 21-23.

Freeman LJ, Rahmani EY, Al-Haddad M, Sherman S, Chiorean MV, Selzer DJ, Snyder PW, Constable PD, 2010: Comparison of pain and postoperative stress in dogs undergoing natural orifice transluminal endoscopic surgery, laparoscopic, and open oophorectomy. *Gastrointest Endosc,* 72 (2), 373-380.

Granados JR, Uson-Casaus J, Martinez JM, Sanchez-Margallo F, Perez-Merino E, 2017: Canine laparoscopic ovariectomy using two 3-and 5-mm portal sites: A prospective randomized clinical trial. *Can Vet J,* 58 (6), 565-570.

Hancock RB, Lanz OI, Waldron DR, Duncan RB, Broadstone RV, Hendrix PK, 2005: Comparison of postoperative pain after ovariohysterectomy by harmonic scalpel-assisted laparoscopy compared to median celiotomy and ligation in dogs. *Vet Surg,* 34, 273-282.

Harris KP, Adams VJ, Fordyce P, Ladlow J, 2013: Comparison of surgical duration of canine ovariectomy and ovariohysterectomy in a veterinary teaching hospital. *J Small Anim Pract,* 54 (11), 579-583.

Howe LM, 2006: Surgical methods of contraception and sterilization. *Theriogenology,* 66, 500-509.

Howe LM, Slater MR, Boothe HW, Hobson HP, Holcom JL, Spann AC, 2001: Long-term outcome of gonadectomy performed at an early age or traditional age in dogs. *J Am Vet Med Assoc,* 218, 217-221.

Hsueh C, Giuffrida M, Mayhew PD, Case JB, Singh A, Monnet E, Holt DE, Cray M, Curcillo C, Runge JJ, 2018: Evaluation of pet owner preferences for operative sterilization techniques in female dogs within the veterinary community. *Vet Surg,* 47 (S1), O15-O25.

Impellizeri JA, Tetrick MA, Muir P, 2000: Effect of weight reduction on clinical signs of lameness in dogs with hip osteoarthritis. *J Am Vet Med Assoc,* 216, 1089-1091.

Manassero M, Viateau V, 2018: Advances in laparoscopic spay techniques for dogs: the past, present and future. *Vet Rec,* 183 (24), 742-744.

Marvel SJ, 2022: Concepts in sterilization, *Vet Clin N Am Small Anim Pract,* 52 (2), 419-436.

Mayhew PD, Brown DC, 2007: Comparison of three techniques for ovarian pedicle hemostasis during laparoscopic-assisted ovariohysterectomy. *Vet Surg,* 36, 541-547.

Moxon R, Freeman SL, Payne R, Corr S, England GCW, 2023: A prospective cohort study investigating the peri- and postoperative outcomes following ovariohysterectomy in bitches neutered prepubertally or post-pubertally. *Theriogenology*, 197, 283-294.

Nylund AM, Drury A, Weir H, Monnet E, 2017: Rates of intraoperative complications and conversion to laparotomy during laparoscopic ovariectomy performed by veterinary students: 161 cases (2010-2014). *J Am Vet Med Assoc,* 251 (1), 95-99.

Öhlund M, Höglund O, Olsson U, Lagerstedt A, 2011: Laparoscopic ovariectomy in dogs: A comparison of the LigaSure™ and the SonoSurg™ systems. *J Small Anim Pract,* 52 (6), 290-294.

Pope JFA, Knowles TG, 2014: Retrospective analysis of the learning curve associated with laparoscopic ovariectomy in dogs and associated perioperative complication rates. *Vet Surg,* 43(6), 668-677.

Stubbs WP, Bloomberg MS, 1995: Implications of early neutering in the dog and cat. *Semin Vet Med Surg (Small Anim),* 10, 8-10

Tallant A, Ambros B, Freire C, Sakals S, 2016: Comparison of intraoperative and postoperative pain during canine ovariohysterectomy and ovariectomy. *Can Vet J,* 57 (7), 741.

Tapia-Araya AE, Martin-Portugues IDG, Sanchez-Margallo FM, 2015: Veterinary laparoscopy and minimally invasive surgery. *Companion Animal,* 20 (7), 382-392.

Tez G, Kanca H, 2018: The use of laparoscopy in veterinary gynecology. *Turkiye Klinikleri J Vet Sci Obstetrics and Gynecology-Special Topics,* 4 (1), 72-80.

Tez G, Kanca H, Alemdar H, 2019: Contraception in dogs I: Evaluation of surgical sterilization risk and benefits. *Vet Hekim Der Derg*, 90 (1), 55-65.

Valdez V, 2022: High volume spay and neuter: a safe and time efficient approach, Elsevier, Philadelphia, USA.

Van Goethem BE, Schaefers-Okkens A, Kirpensteijn J, 2006: Making a rational choice between ovariectomy and ovariohysterectomy in the dog: A discussion of the benefits of either technique. *Vet Surg,* 35, 136-143.

Van Goethem BE, Rosenveldt KW, Kirpensteijn J, 2003: Monopolar vs. bipolar electrocoagulation in canine laparoscopic ovariectomy: A nonrandomized, prospective, clinical trial. *Vet Surg,* 32, 464-470.

Van Nimwegen SA, Kirpensteijn J, 2007: Comparison of Nd:YAG surgical laser and Remorgida bipolar electrosurgery forceps for canine laparoscopic ovariectomy. *Vet Surg*, 36, 533-540.

Van Nimwegen Sa, Van Swol CFP, K Kirpensteijn J, 2005: Neodymium: Yttrium aluminum garnet surgical laser versus bipolar electrocoagulation for laparoscopic ovariectomy in dogs. *Vet Surg,* 34 (4), 353-357.

HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Initial Clinical Manifestations of Dogs with Neurological Distemper

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Abstract: The canine distemper virus (CDV) causes demyelination within the central nervous system, gastrointestinal and/or respiratory signs. Due to the wide variety of clinical manifestations, determining the initial clinical manifestation of dogs with neurological CDV of different ages can be used to increase the index of suspicion of CDV, especially in triage. 44 dogs, aged 2-8 months, with clinical findings suggesting the presence of neurological CDV were used, and 38 were enrolled. The dogs were divided into 2 subgroups based on their age. Accordingly, dogs aged 2-4 months were included in Group 1 (n:16), and dogs aged 5-8 months were included in Group 2 (n:22). The body temperature of Group 1 was higher than that of Group 2 ($p<0.000$). Within the scope of extraneural findings, fever, anorexia, depression, ocular discharge, nasal discharge, diarrhea, uveitis, and pneumonia were detected. Neural findings included behavioral change, head tilt, quadriplegia, convulsion, ataxia and myoclonus. Thoracic radiography revealed a cranioventral alveolar pulmonary pattern and diffuse interstitial pneumonia. The presence of the foreign body in the gastrointestinal tract was prominent in Group 2. On thoracic ultrasonography, B lines (>3 lines) were prominent in both groups. Abdominal ultrasonography revealed dilated intestine segments. It was concluded that the evaluation of the initial clinical manifestation in combination with non-invasive imaging methods might be used to increase the index of suspicion of neurologic CDV before proceeding to laboratory analyses, especially in triage.

Keywords: *Diagnosis, Dog, Radiography, Ultrasound.*

Nörolojik Distemperli Köpeklerin Başlangıç Klinik Görünümü

Özet: Kanin distemper virus (CDV), merkezi sinir sistemi içinde demyelinizasyona, gastrointestinal ve/veya respiratorik semptomlara yol açabilir. Çok çeşitli klinik bulguları nedeniyle, farklı yaştaki nörolojik CDV'li köpeklerin başlangıç klinik görünümlerinin belirlenmesi, özellikle triyajda, CDV şüphesi indeksini artırmak için kullanılabilir. Klinik bulguları nörolojik CDV varlığından şüphelendiren 2-8 aylık 44 köpeğin 38'i çalışmaya dahil edildi. Köpekler yaşlarına göre iki alt gruba ayrıldı. Buna göre, 2-4 aylık köpekler Grup 1'e (n:16), 5-8 aylık köpekler Grup 2'ye (n:22) dahil edildi. Grup 1'in vücut sıcaklığı Grup 2'den yüksekti ($p<0.000$). Ekstranöral bulgular kapsamında ateş, iştahsızlık, depresyon, göz ve burun akıntısı, diyare, üveit ve pnömoni belirlendi. Nöral bulgular arasında davranış değişikliği, kafa sallama, kuadripleji, konvülsiyon, ataksi ve miyoklonus belirlendi. Toraks radyografisinde kranioventral alveoler pulmoner patern ve yaygın interstisyel pnömoni saptandı. Grup 2'de, gastrointestinal sistemde yabancı cisim varlığı dikkat çekiciydi. Toraks ultrasonografisinde her iki grupta da B çizgileri (>3 çizgi) belirgindi. Abdominal ultrasonografide dilate barsak segmentleri gözlendi. İnvaziv olmayan görüntüleme teknikleri ile birlikte başlangıç klinik görünümün değerlendirilmesinin, özellikle triyajda, laboratuvar analizlerine geçmeden önce nörolojik CDV şüphe indeksini artırmak için kullanılabileceği kanısına varıldı.

Anahtar Kelimeler: *Köpek, Radyografi, Tanı, Ultrasonografi.*

Introduction

In the field of veterinary neurology, various etiologies such as metabolic, degenerative, nutritional, autoimmune, inflammatory and infectious can affect the brain and cause encephalopathy (Dewey, 2008). Canine distemper virus (CDV), which has an important place among infectious encephalopathies of dogs, is a re-emerging agent that can cause an epidemic even in vaccinated dogs worldwide (Lan et al., 2006). In addition, diagnosing CDV-associated encephalomyelitis is clinically difficult without traditional findings such as systemic manifestations (Amude et al., 2012). Systemic findings associated with CDV are loss of appetite, fever, nasal/ocular discharge, cough, dyspnea, vomiting and diarrhea. These findings may be in combination with each other and neurological findings may occur without systemic findings (Tipold et al., 1992). In previous studies, it was reported that conjunctivitis, fever, respiratory and gastrointestinal system-related extraneural findings were detected in two-thirds of the dogs admitted to the hospital, while no extraneural findings were detected in one-third (Amude et al., 2012; Tipold et al., 1992).

Myoclonus is a characteristic and typical sign in dogs with CDV-related encephalomyelitis. However, the nervous form of CDV infection may occur without myoclonus and systemic involvement. Although there are many studies on dogs with CDV-related neuropathy (Amude et al., 2012; Appel, 1994; Gülersoy et al., 2022), data evaluating the clinical syndromes of CDV infection are available only in classical textbooks or reviews (Amude et al., 2012; Braund, 1994). The number of studies evaluating the whole clinical manifestation is limited.

The present study aimed to determine the initial clinical manifestation of dogs of different ages with clinical findings that would suggest neurological CDV infection, and to determine which clinical findings could be used to increase the index of suspicion of CDV in triage.

Material and Methods

This study protocol was approved by the ethics committee of the Faculty of Veterinary Medicine, Harran University (session and permit number: 2021-005/01-14). This article was produced from the study entitled Evaluation of the Efficacy of Blood Gases and Hemogram Parameters in the Diagnosis of non-Neurogenic Distemper and Parvoviral Enteritis in Dogs with Acute Gastroenteritis.

Animal Material: This study consisted of 44 dogs, aged 2-8 months, with clinical findings such as respiratory and gastrointestinal signs, fever and neurologic disorders that would suggest CDV infection. All animals were client-owned (30 males, 14 females; 38 mix breed, 3 Labrador, 2 Rotweiler, 1 Pointer) and admitted to Harran University Faculty of Veterinary Animal Hospital for diagnostic and/or treatment purposes. The owner's consent was obtained before the clinical evaluation and sample collection.

Anamnestic Data: None of the dogs were neither vaccinated nor dewormed. All animals were fed on commercial dry dog food. Of the dogs deemed suitable for inclusion in the study, 24 were outdoor, and 20 were indoor dogs. The mean duration of clinical symptoms of all the dogs was 8 (3-22) days.

Clinical Examinations: Body temperature, gingival capillary refill time, evaluation of palpable lymph nodes, as well as heart and lung auscultation were measured for all dogs eligible for inclusion in the study. Also, fecal samples were obtained from all the dogs with sterile swabs to microscopically investigate the presence of parasites (light microscope, x40 magnification, light microscope, Olympus®). Findings detected as a result of physical examinations were classified as extraneural and neural. Thoracic/abdominal radiographic (Fujifilm VXR, Japan) and ultrasonographic (Mindray Z60, China) examinations were performed following the physical examination.

The imaging depth for ultrasonographic examination of the thorax was set at 3–6 cm depending on the animal. The focus position was set to the pleural line. All examinations were performed in sternal positions or standing without clipping, after the application of alcohol and coupling gel on the chest. Multiple scan fields were evaluated for a complete examination of the thorax (using 8 MHz microconvex probe). The patient was placed in dorsal recumbency for the abdominal ultrasonography and gently restrained. Cranioventral abdominal hair was clipped and wetting the skin with water, a tincture of 70% isopropyl alcohol, followed by ultrasound gel that permits obtaining an improved image (using 6-8 MHz microconvex probe).

Blood Sampling and Application of Rapid Diagnostic Test Kits: To eliminate any diseases causing similar symptoms CDV, Canine Adenovirus 2, Canine Influenza virus, Canine Coronavirus (Asan Easy Test CAV2/CIV/CCV Ag, ASAN Pharm. Co., Ltd. Gyeonggi-do Korea, relative sensitivity: 93.10%, relative specificity: 97.50%) and Canine Parvovirus antigen tests (Asan Easy Test CPV Ag, ASAN Pharm. Co., Ltd. Gyeonggi-do Korea, relative sensitivity: 97.96%, relative specificity: 97.50%) were performed according to the manufacturer's instructions. All results were negative. CDV Ag test (Asan Easy Test CDV Ag, ASAN Pharm. Co., Ltd. Gyeonggi-do Korea, relative sensitivity: 97.96%, relative specificity: 97.50%) was performed by using both ocular and nasopharyngeal secretions were obtained with sterile wet swabs according to the manufacturer's instructions to confirm suspicion of CDV infection.

Inclusion/Exclusion Criteria: Dogs with clinical findings that would suggest CDV infection at first admission, whose clinical findings were classified as extraneural and neural, with a positive CDV Ag and a negative CAV2/CIV/CCV test result, were included in the study. Dogs with any concurrent disease or with parasites/parasite eggs in feces were not included. As a result, 38 out of 44 dogs with CDV-related clinical findings were enrolled in the study.

Forming Subgroups: 38 dogs, whose CDV infection was confirmed by clinical and rapid diagnostic test kits were divided into 2 subgroups based on age. Accordingly, dogs aged 2-4 months were included in Group 1 (n:16), and dogs aged 5-8 months were included in Group 2 (n:22).

Statistical Analysis: The data were evaluated using statistical software (SPSS 25.00 for Windows). One sample Kolmogorov-Smirnov test was applied to determine whether all data were non-parametric or parametric. Non-parametric data were presented as median (min, max) with Mann Whitney U, Kruskal-Wallis test. Statistical significance was considered as $p<0.05$ for all data.

Results

Physical Examination Findings: As a result of physical examinations, the body temperature of Group 1 was higher than that of Group 2 ($p<0.000$). Physical examination findings are presented in Table 1. In Group 1, within the scope of extraneural findings, fever (8 out of 16, 50%), anorexia (14 out of 16, 87.5%), depression (15 out of 16, 93.75%), ocular discharge (13 out of 16, 81.25%), nasal discharge (13 out of 16, 81.25%), diarrhea (5 out of 16, 31.25%), uveitis (5 out of 16, 31.25%) and pneumonia (10 out of 16, 62.5%) were detected. Neural findings included behavioral change (14 out of 16, 87.5%), head tilt (10 out of 16, 62.5%), quadriplegia (5 out of 16, 31.25%), convulsion (8 out of 16, 36.36%), ataxia (5 out of 16, 31.25%) and myoclonus (15 out of 16, 93.75%). The distribution of clinical symptoms of Group 1 is presented in Figure 1. Extraneural findings of Group 2 were determined to be fever (8 out of 22, 36.36%), anorexia (16 out of 22, 72.72%), depression (18 out of 22, 81.81%), ocular discharge (16 out of 22, 72.72%), nasal discharge (15 out of 22, 68.18%), diarrhea (14 out of 22, 63.63%), uveitis (13 out of 22, 59.09%), hardpad (1 out of 22, 4.54%), and pneumonia (11 out of 22, 50%). Within scope of neural findings of Group 2, behavioral change (14 out of 22, 63.63%), head tilt (10 out of 22, 45.45%), quadriplegia (5 out of 22, 22.72%), convulsion (8 out of 22, 36.36%), ataxia (5 out of 22, 22.72%) and myoclonus (15 out of 22, 68.18%) were detected. The distribution of clinical symptoms of Group 2 is presented in Figure 2.

Table 1. Physical examination findings.

Clinical Parameters	Group 1 (n:16) median (min-max)	Group 2 (n:22) median (min-max)	p value
Symptom duration (days)	6.5 (3-19)	9 (3-22)	0.140
Body weight (kg)	7.85 (5.3-11.2)	10.75 (7-16)	0.000
Body temperature (°C)	39.25 (38.8-39.8)	38.45 (36.6-39.6)	0.000
Capillary refill time (sec)	2 (1-3)	2 (1-4)	0.071
Respiration rate (breath/min)	69 (44-93)	89 (46-99)	0.189
Heart rate (beat/min)	101.5 (68-124)	86 (65-144)	0.101

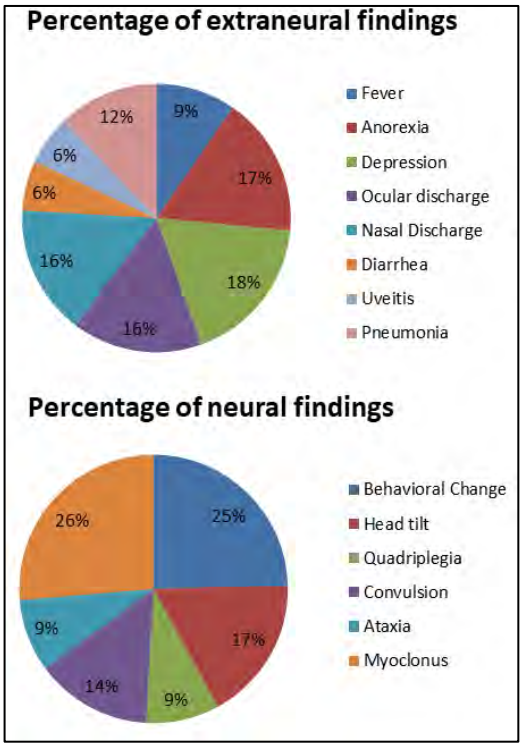


Figure 1. Distribution of extraneural and neural clinical symptoms of Group 1.

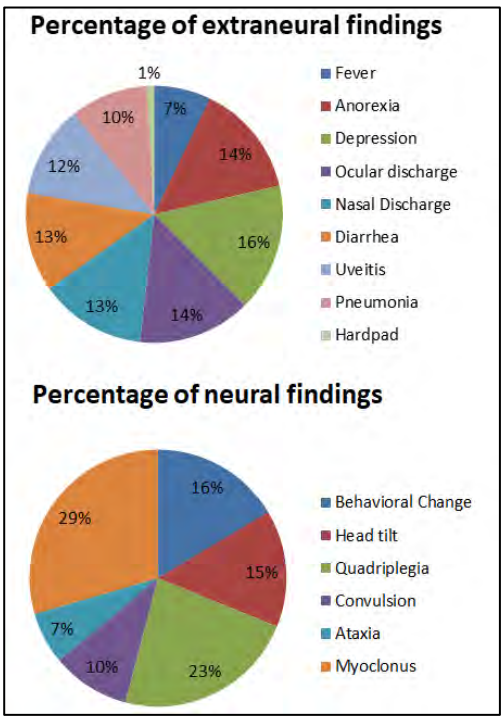
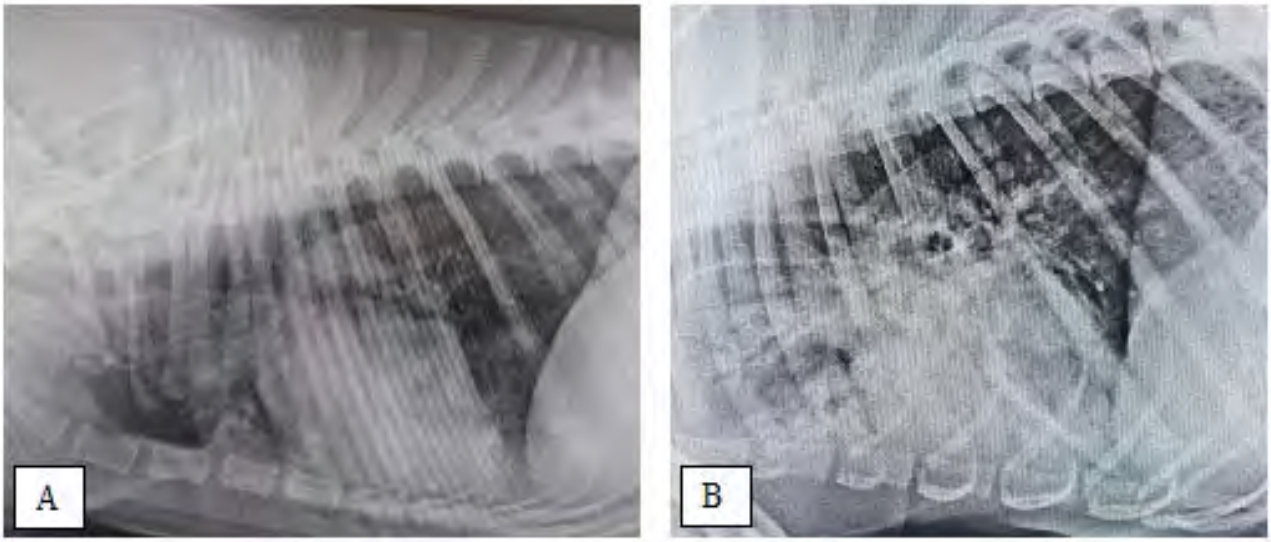


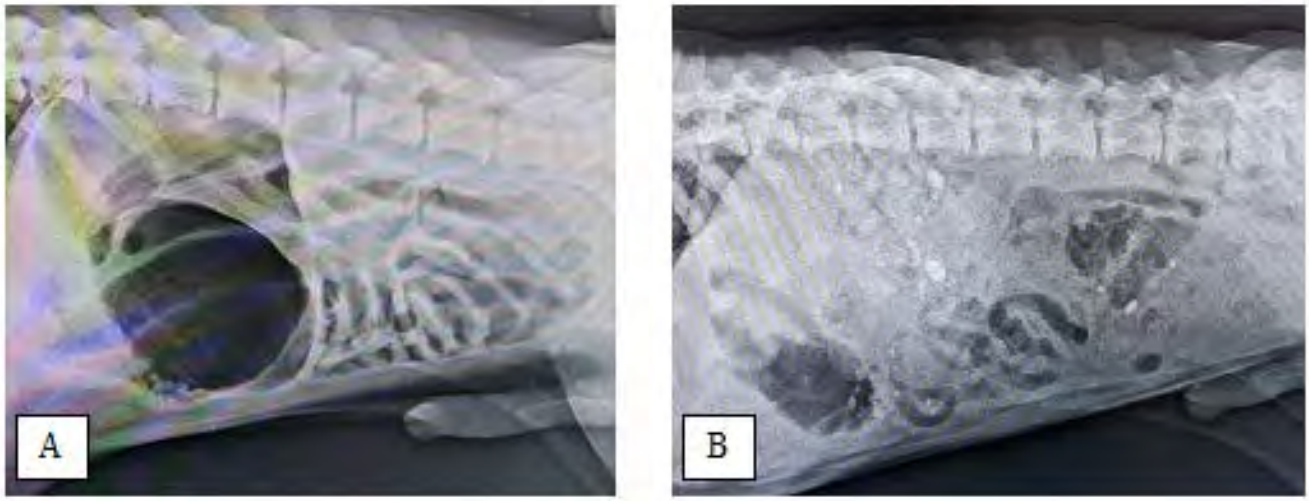
Figure 2. Distribution of extraneural and neural clinical symptoms of Group 2.

Radiographic Findings: Thoracic radiography revealed a cranioventral alveolar pulmonary pattern (4 out of 16, 25%), diffuse interstitial pneumonia (5 out of 16, 31.25%), and enlarged mediastinal lymph node (3 out of 16, 18.75%) in Group 1. In Group 2, caudodorsal alveolar pattern (9 out of 22, 40.99%) and diffuse interstitial pneumonia (10 out of 20, 45.45%) were evident. Abnormal thoracic radiographic findings are presented in Figure 3. According to the abdominal radiographs, intestinal distension (11 out of 16, 68.75%) was evident in Group 1. In contrast, gas and dilated stomach (8 out of 22, 36.36%) and foreign body in the gastrointestinal tract (18 out of 22, 81.81%) were prominent in Group 2. Abnormal abdominal radiograph findings are presented in Figure 4.



A. Alveolar pattern, which is characterized by the existence of more or less broad portions of the lung more opaque than normal due to partial or complete alveolar filling. B. Interstitial pneumonia, which is characterized by lung volume loss and an apicobasal gradient of peripheral septal thickening and bronchiectasis.

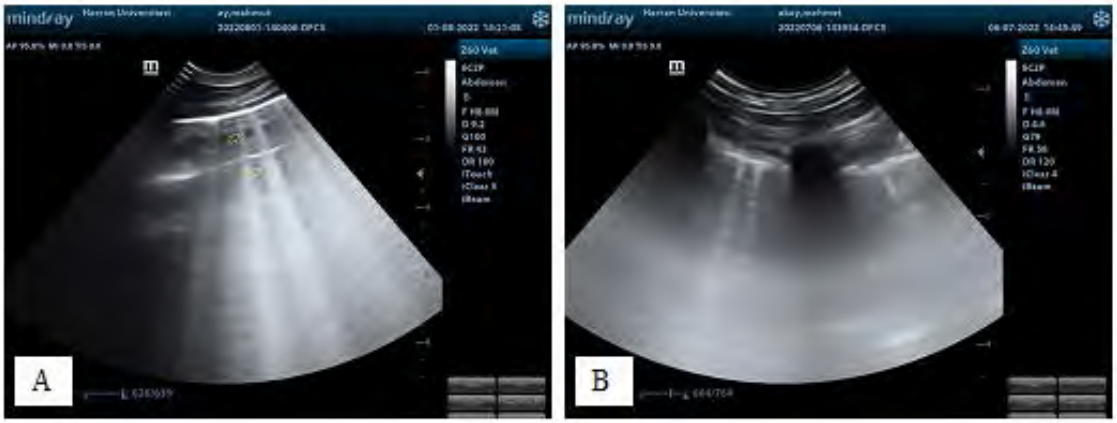
Figure 3. Abnormal thoracic radiographic findings.



A. Gas-filled stomach, and intestinal distention which is characterized by multiple, small, randomly distributed, gaseous foci scattered throughout the abdomen. B. Intestinal distension, and foreign body causing radio-opaque appearance in the intestines.

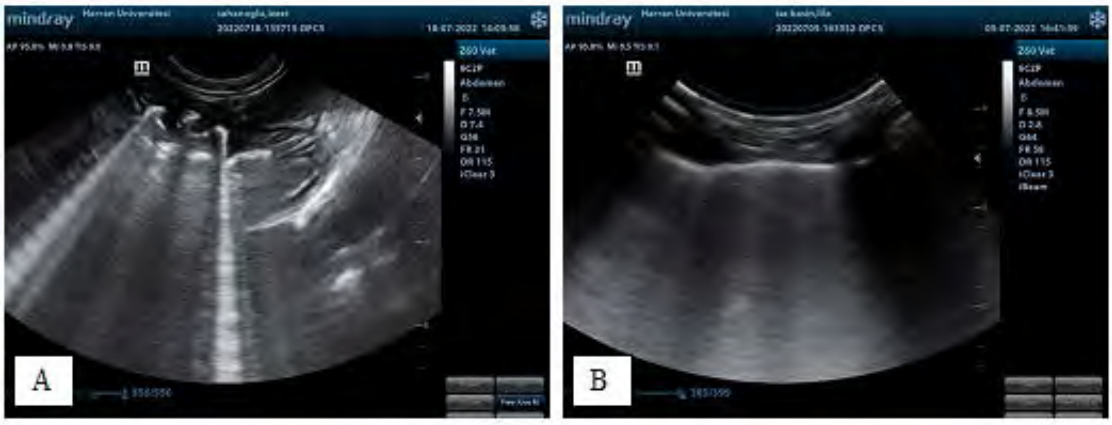
Figure 4. Abdominal radiographic findings detected.

Ultrasonographic Findings: On thoracic ultrasonography, B lines (>3 lines) were prominent in both groups (Group 1, 12 out of 16, 75%; Group 2, 14 out of 22, 63.63%). In Group 2, the loss of the A lines (5 out of 22, 22.72%) was also evident. Lung sliding was detected in both groups. Abnormal thoracic ultrasonography findings are presented in Figure 5. On the other hand, in abdominal ultrasonography, dilated intestine segments with gas (10 out of 16, 62.5%) were detected in Group 1, while acoustic shadowing, which is characterized by a clear shadow that suspects the presence of a foreign body in the stomach and intestines was evident in Group 2 (17 out of 22, 77.27%). Abnormal abdominal ultrasonography findings are presented in Figure 6.



A. Multiple B lines origination from pleura. B. Loss of A lines along with the presence of B lines which is also known as comet-tail artifact indicating subpleural interstitial edema.

Figure 5. Abnormal thoracic ultrasonography findings.



A. Clear acoustic shadow indicating the presence of a foreign body in the stomach. B. Dirty shadow associated with the presence of gas in the intestines.

Figure 6. Abnormal abdominal ultrasonography findings.

Discussion

In this study, the initial clinical manifestations and imaging results of dogs with neurological CDV were evaluated in triage, and important findings were achieved. While the incidence of extraneural findings such as diarrhea, uveitis and neural findings such as quadriplegia were lower in 2-4 month-old dogs, the same findings had a higher incidence in 5-8 month-old dogs. Non-specific findings such as depression and anorexia were the most critical extraneural findings in both groups whereas myoclonus and behavioral change were the most prominent neural findings. In accordance with these results, it was concluded that the evaluation of initial clinical manifestation with non-invasive imaging methods such as radiography and ultrasonography could be used to increase the index of suspicion of neurological CDV before proceeding to laboratory analyses such as complete blood count, serum biochemistry and rapid diagnostic test applications, especially in triage.

CDV causes a progressive, multifocal demyelinating disease within the central nervous system. CDV infection may also lead to gastrointestinal and/or respiratory signs (Amude et al., 2006). After the entrance of the virus into the body via nasal or oral route, it rapidly replicates in the lymphoid tissue and causes immunosuppression. Transient fever peaks 3-6 days after infection and during this period, loss of appetite, depression, ocular and nasal discharges and tonsillitis can be detected. 6-9 days after infection, the virus spreads to the epithelium of most organs with cell-associated viremia (Appel et al., 1982; Winters et al., 1984). At this stage, the severity of the symptoms depends on the virulence of the virus strain, the immune status and the age of the dog. If the dog has a weak immune response, the virus attains epithelial cells and the central nervous system (CNS). The initial clinical findings disappear; the virus persists for long periods in the neurons, uvea, urothelium and soles of the feet (hard pad). If the dog shows no immune response, the virus continues to replicate and spreads throughout the body, and usually becomes more severe with secondary bacterial infections (Green and Appel, 1990). Neurological symptoms such as head tilt, circling, nystagmus, complete or partial paralysis, convulsions, behavioral changes and dementia begin 20 days after the onset of infection. Involuntary muscle contractions, twitching and gum chewing

are considered typical symptoms for CDV infection. In some dogs, neurologic signs may take up to 40-50 days (Green and Appel, 1990; Vandevalde and Zurbriggen, 2005). Considering the duration of symptoms of naturally CDV-infected dogs of the present study (8 (3-22) days), the wide range of neural and extraneural findings, including gastrointestinal and respiratory symptoms, can be attributed to the properties of CDV's antigenic recognition by hemagglutinin protein (H), SLAM and nectin-4-mediated lymphoid tissue interaction and multiple cell tropism (Rendon-Marin et al., 2019). In addition, the higher body temperature detected in Group 1 compared to Group 2 was associated with the duration of onset of infection (Table 1) and incomplete immune maturation of the puppies (Handl et al., 2009).

Considering variables such as the type of neurological signs, the presence of concomitant systemic disease, age, vaccination and immunity status of the dog, and the frequency of neurological signs, five neurological syndromes associated with CDV were identified. These are: canine distemper encephalomyelitis in immature dogs (CDEID), multifocal distemper encephalomyelitis in mature dogs (MDEMD), chronic relapsing distemper encephalomyelitis (CRDE), post vaccinal distemper encephalomyelitis (PVDE) and old dog encephalomyelitis (ODE) (Headley et al., 2009). In acute cases, primary demyelination is not associated with inflammation due to the absence of prominent perivascular cuffs, reduced myelin synthesis in CDV-infected oligodendrocytes and metabolic dysfunction and activation of microglia cells (Vandevalde and Zurbriggen, 2005). In chronic cases, demyelination is associated with inflammation triggered by CDV-specific immune response (Griot et al., 1989). In ODE-like encephalitis, which is a rare form, progressive cortical derangement with multifocal perivascular and parenchymal lymphoplasmacytic encephalitis is evident in the cerebral hemispheres (Plattet et al., 2005). Diagnosing CDV-associated encephalomyelitis is difficult in the absence of CDV-specific neurologic findings such as myoclonus with respiratory/gastrointestinal signs. It was reported that chewing-gum movement in CDV cases is due to contraction of facial and masticar muscles and generalized partial seizures are due to tonic-clonic movements of all skeletal muscles. In addition, behavioral changes are reported to be associated with cerebral dysfunction in dogs with CDEID. Additional findings reported in this context include loss of consciousness, blindness and spastic tetraparesis (Braund, 1994). Loss of consciousness and behavioral changes have been associated with ascending reticular activating system (ARAS) dysfunction in the brain stem (Dewey, 2008). Nevertheless, the neurological signs are related to the affected area of the brain (Braund, 1994; Vite, 2005). The involvement of cortical and subcortical regions of the brain can explain the behavioral changes of the present study. In contrast, the loss of consciousness finding can be explained by the involvement of the brain stem (Vite, 2005). The lower incidence of findings, such as quadriplegia and ataxia, may be related to the rarer spinal cortex damage in dogs with CDEID (Braund, 1994). Although previous studies reported that gastrointestinal signs such as vomiting, diarrhea and respiratory signs such as cough are frequently observed in dogs with CDVID (Vite, 2005), diarrhea was found to be a less common finding in the present study, especially in 2–4-month-old dogs. Diarrhea, which has a relatively higher incidence in 5–8-month-old dogs, and anorexia, when evaluated with other compatible findings, can be used to increase the CDV suspicion index in older dogs.

CDV affects both gray and white matter in the CNS. For this reason, neurological findings vary and seizures, behavioral changes, cerebellar (cranial and truncal ataxia, hypermetria, tremor), vestibular (falling, head tilt, rotation and nystagmus) and visual disturbances, paresis, paralysis, myoclonus and limb weakness may be observed (Amude et al., 2007). Seizures and chewing gum fits are associated with the region where the cerebellum is affected (Greene and Appel, 1998). Myoclonus is the rhythmic twitching of a single or group of muscles. It is a characteristic finding of neurological CDV in young dogs (Braund, 1994). However, it has also been reported to occur episodically in other inflammatory diseases in which the CNS is affected (Tipold et al., 1992). The lower motor neurons of the spinal cord's and cranial nerve nuclei's degenerative alterations are thought to cause the myoclonus. These rhythmic muscle contractions are caused by this lesion, which develops an independent pacemaker. However, pathological changes are minimal in these gray matter regions suggesting afunctional problems may be implicated (DeLahunta and Glass, 2009). In a clinicopathological analysis, no lesions in the neural gray column of the related spinal cord segments were observed in 5 out of 13 spontaneous distemper patients with myoclonus (Koutinas et al., 2002). In the present study, myoclonus was the neural sign with the highest incidence in both groups. This sign may be related to pathologic changes of the cranial nerve nucleus in younger dogs and of the spinal cord in older dogs, considering the high incidence of quadriplegia.

Ataxia is the most common finding in dogs with MDEMD, with paresis, especially in the hind limbs (Shell, 1990). MDEMD is defined as multifocal encephalomyelitis with a chronic course and lower incidence. In this form of CDV, the cerebellum, brainstem and spinal cord are the affected areas (Braund, 1994). Spastic paraplegia, truncal or head tilt, dysmetria and hypermetria have been reported as common findings when the CNS is affected with multifocal lesions (Amude et al., 2007). The head tilt in the 2–4-month-old dogs of the present study may be associated with multifocal pathologic changes of the cerebellum, although the cases were not identified as MDEMD.

Data on behavioral changes in neurological CDV cases is limited. In a previous study, findings such as aggression, head leaning against objects, circling, altered state of consciousness, unconscious gait, and interest in foreign objects were reported within the scope of behavioral changes. These findings were defined as ODE-like syndrome and reported to occur due to progressive panencephalitis with a rare subacute-chronic course (Shell, 1990; Vite, 2005). In addition, a low incidence of spinal cord-related findings, such as ataxia and quadriplegia, were reported in dogs with ODE-like syndrome (Braund, 1994). In all groups of the

present study, the highest incidence of neural findings was behavioral change, and Group 1, which consists of younger dogs, also had a lower incidence of findings associated with spinal cord injury. Within the scope of the behavioral changes determined in the present study, barking at night, interest in foreign objects, head leaning, and aggression were learned from the anamnestic data. In addition, radiographic and ultrasonographic examinations of all dogs revealed the presence of bones and stones in the stomach and intestines (appearance characterized by a clear shadow on ultrasound, which is suspicious for the presence of a foreign body.). The ingestion of materials such as stones, plastic, rope, wood, etc., termed pica by the animal, does not provide any physical benefit and may be associated with some medical, nutritional and behavioral disorders. The higher presence of gastrointestinal foreign bodies in puppies (aged 2-4 months) included in Group 1 of the present study, combined with etiologic factors such as teething, anxiety and lack of enrichment (Masson et al., 2021), may be used to consider the presence of panencephalitis in combination with other CDV compatible findings and to increase the index of suspicion for neurologic CDV.

Following epithelial localization, the virus is known to cause respiratory symptoms such as pneumonia and gastrointestinal symptoms such as diarrhea (Green and Appel, 1990). Previous studies reported a general, moderate, slightly heterogeneous increase in pulmonary opacity, multiple thickened bronchial ring shadows, and decreased delineation of peripheral pulmonary vessels within the thoracic radiographic findings of dogs with CDV (Willi et al., 2015). Sonographic evaluation of the thoracic and abdominal cavities provides diagnostic information. Thoracic radiographic findings, such as alveolar pulmonary pattern and diffuse interstitial pneumonia, and thoracic ultrasonographic findings, such as the presence of B lines (>3 lines) along with loss of A-lines in the present study were compatible with the presence of pneumonia (Mazzola et al., 2021). Abdominal radiographic findings such as intestinal distention and gas-filled stomach, together with abdominal ultrasonographic findings such as acoustic shadowing characterized by a clean shadow in the intestine may be related to the aerophagia as a result of dyspnea due to pneumonia, and foreign body affinity due to abnormal mental status in dogs with CDV (Harjes et al., 2018).

The fact that the neurological findings determined in the present study were not histopathologically demonstrated and that other etiologies of the presence of gastrointestinal foreign body associated with behavioral changes, which is among the important clinical findings, were not investigated, can be considered as limitations. Therefore, investigating hematologic, biochemical and trace element concentrations in dogs with neurologic CDV with the aforementioned clinical findings may reveal predisposing factors as well as disease pathogenesis.

Conclusion

The clinical diagnosis of neurologic CDV infection applies to traditional cases. Cases without traditional symptoms require further diagnostic methods. Therefore, evaluation of the initial clinical manifestation allows early diagnosis and thus, treatment of CDV cases. In the present study, extraneural findings such as diarrhea and uveitis, and neural findings such as quadriplegia had a higher incidence in 5-8 month-old dogs; depression, anorexia, myoclonus, and behavioral changes along with the presence of gastrointestinal foreign body had a high incidence in all the dogs. It was concluded that the evaluation of the initial clinical manifestation in combination with non-invasive imaging methods such as radiography and ultrasonography could be used to increase the index of suspicion of neurologic CDV before proceeding to laboratory analyses, especially in triage.

Conflict of Interest

The authors stated that they did not have any real, potential, or perceived conflict of interest.

Ethical Approval

Permission was received for this study under HADYEK number 2021-005/01-14. Additionally, the authors declared that Research and Publication Ethics were complied with.

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References

Amude AM, Alfieri AA, Alfieri AF, 2006: The nervous form of canine distemper. *Vet e Zootec,* 13 (2), 125-136.

Amude AM, Alfieri AA, Arias MVB, Alfieri AF, 2012: Clinical syndromes of nervous distemper in dogs initially presented without conventional evidences of CDV infection. *Semin Ciênc Agrár*, 33 (6), 2347-2358.

Amude AM, Alfieri AA, Alfieri AF, 2006: Antemortem diagnosis of CDV infection by RT-PCR in distemper dogs with neurological deficits without the typical clinical presentation. *Vet Res Commun*, 30 (6), 679.

Appel MJ, Mendelson SG, Hall WW, 1984: Macrophage Fc receptors control infectivity and neutralization of canine distemper virus-antibody complexes. *J Virol*, 51 (3), 643–649.

Appel MJ, Shek WR, Summers BA, 1982: Lymphocyte-mediated immune cytotoxicity in dogs infected with virulent canine distemper virus. *Infect Immun*, 37 (2), 592–600.

Appel MJ, Yates RA, Foley GL, Bernstein JJ, Santinelli S, Spelman LH, Miller LD, Arp LH, Anderson M, Barr M, Pearce-Kelling S, Summers BA, 1994: Canine distemper epizootic in lions, tigers, and leopards in North America. *J Vet Diagn*, 6 (3), 277-288.

Braund, 1994 Braund KG, Toivio-Kinnucan M, Vallat JM, Mehta JR, Levesque DC, 1994: Distal sensorimotor polyneuropathy in mature Rottweiler dogs. *Vet Pathol*, 31 (3), 316-326.

Braund KG, 1994: Distemper. In: Clinical Syndromes in Veterinary Neurology. Braund KG (Ed), 115-119, St Louis, Mosby.

DeLahunta A, Glass E, 2009: Upper motor neuron. In: Veterinary Neuroanatomy and Clinical Neurology, DeLa-hunta A, Glass E (Eds). 192-229, Elsevier Health Science, St Louis, MO, USA.

Dewey CWA, 2008: Pratical Guide To Canine and Feline Neurology. 2nd ed. Wiley-Blackwell, Ames, Iowa, USA.

Green EC, Appel MJ, 1990: Canine distemper virus. In: Infections diseases of the dog and cat. Greene CE (Ed), 226-241. WB Saunders Company, Philadelphia, USA.

Griot C, Burge T, Vandevelde M, Peterhans E, 1989: Antibody-induced generation of reactive oxygen radicals by brain macrophages in canine distemper encephalitis: a mechanism for bystander demyelination. *Acta Neuropathol,* 78, 396–403.

Gülersoy E, Balıkçı C, Günal İ, Şahan A, 2022: Evaluation of the Efficacy of Blood Gases and Hemogram Parameters in the Diagnosis of non Neurogenic Distemper and Parvoviral Enteritis in Dogs with Acute Gastroenteritis. *Rev Cient Fac Cienc Vet*, 32.

Handl S, Wehr U, Zentek J, Krammer-Lukas S, 2009: Histological and immunohistochemical evaluation of duodenal and colonic biopsies after oral bovine lactoferrin supplementation in beagle puppies. *J Anim Physiol Anim Nutr*, 93, 76–82. doi: 10.1111/j.1439-0396.2007.00781.x

Harjes L, Le Pommellet H, Hostnik E, Rudinsky A, 2018: Resolution of esophageal dysmotility following treatment of nasal disease in a dog. *Can Vet J*, 59 (2), 147-151.

Headley SA, Amude AM, Alfieri AF, Bracarense APF, Alfieri AA, Summers BA, 2009: Molecular detection of canine distemper virus and the immunohistochemical characterization of the neurologic lesions in naturally occurring old dog encephalitis. *J Vet Diagn Invest*, 21 (5), 588-597.

Koutinas AF, Polizopoulou ZS, Baumgaertner W, Lekkas S, Kontos V, 2002: Relation of clinical signs to pathological changes in 19 cases of canine distemper encephalomyelitis. *J Comp Pathol*, 126 (1), 47-56.

Lan NT, Yamaguchı R, Inomata A, Furuya Y, Uchıda K, Sugano S, Tateyama S, 2006: Comparative analyses of canine distemper viral isolates from clinical cases of canine distemper in vaccinated dogs. *Vet Microbiol*, 115 (13), 32-45.

Masson S, Guitaut N, Medam T, Béata C, 2021: Link between Foreign Body Ingestion and Behavioural Disorder in Dogs. *J Vet Behav*, 45, 25-32.

Mazzola M, Pugliese NR, Zavagli M, De Biase N, Bandini G, Barbarisi G, D'Angelo G, Sollazzo M, Piazzai C, David S, Masi S, Moggi-Pignone A, Gargani L, 2021: Diagnostic and Prognostic Value of Lung Ultrasound B-Lines in Acute Heart Failure With Concomitant Pneumonia. *Front Cardiovasc Med*, 8, 693912.

Plattet P, Rivals JP, Zuber B, Brunner JM, Zurbriggen A, Wittek R, 2005: The fusion protein of wild-type canine distemper virus is a major determinant of persistent infection. *Virol*, 337 (2), 312–326.

Rendon-Marin S, da Fontoura Budaszewski R, Canal CW, Ruiz-Saenz J, 2019: Tropism and molecular pathogenesis of canine distemper virus. *Virol J*, 16 (30) 1-15.

Shell LG, 1990: Canine distemper. *Compend Contin Educ Vet*, 12 (2), 173-179.

Tipold A, Vandevelde M, Jaggy A, 1992: Neurological manifestation of canine distemper virüs infection. *J Small Anim Pract*, 338 (10): 466-470.

Vandevelde M, Zurbriggen A, 2005: Demyelination in canine distemper virus infection: a review. *Acta Neuropathol*, 109, 56–68.

Vite C, 2005: Inflammatory diseases of the central nervous system. In: Braund's Clinical Neurology in Small Animals: Localization, Diagnosis and Treatment. K. Braund (Ed). International Veterinary Information Service, Ithaca, NY, USA.

Willi B, Spiri AM, Meli ML, Grimm F, Beatrice L, Riond B, Bley T, Jordi R, Dennler M, Hofmann-Lehmann R, 2015: Clinical and molecular investigation of a canine distemper outbreak and vector-borne infections in a group of rescue dogs imported from Hungary to Switzerland. *BMC Vet Res*, 11, 154.

Winters KA, Mathes LE, Krakowka S, Olsen RG, 1984: Immunoglobulin class response to canine distemper virus in gnotobiotic dogs. *Vet Immunol Immunopathol,* 5 (2), 209–215.

HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Incidence of Foot Diseases in Beef Cattle in Kirikkale Region

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Abstract: One of the significant economic losses in cattle farming is associated with hoof diseases. It has been scientifically proven that the incidence of hoof diseases increases in intensive farming operations. This study aims to investigate the incidence of hoof diseases in beef cattle in Kirikkale and its surrounding region. A total of 3047 head of beef cattle, representing different breeds and ages, were examined for hoof and claw deformities. The animals were examined for hoof diseases during specific months of the year by visiting the farms. The study revealed 53 animals with splayed hooves, 1 with an overgrown hoof, 1 with a separated hoof, 1 with a scissor-like hoof, and 1 with a double sole formation. As for hoof diseases, 2 animals had interdigital dermatitis, 8 had digital dermatitis, 10 had heel erosion, 21 had sole ulcers, 5 had interdigital phlegmon, 4 had white line disease, and various forms of laminitis were observed in 22 animals. Considering the barn flooring, management conditions, and intensive farm feeding, it was determined that barn hygiene and hoof care were inadequate. As a result, the impact of management factors specific to the farms, such as barn flooring and ration composition, varied depending on the breed's susceptibility or resistance. Based on the statistical data obtained in conjunction with these factors, the incidence of foot diseases in beef cattle in the Kirikkale region was determined to be 4.23%.
Keywords: *Beef cattle, Foot diseases, Kirikkale province, Incidence.*

Kırıkkale ve Yöresinde Bulunan Besi Sığırlarında Ayak Hastalıklarının İnsidansı

Özet: Sığır yetiştiriciliğinde önemli ekonomik kayıplardan biride ayak hastalıklarına bağlı olarak oluşan kayıplardır. Özellikle entansif yetiştiricilik yapılan işletmelerde ayak hastalıklarının arttığı bilimsel olarak kanıtlanmış gerçeklerdendir. Bu çalışmada, Kırıkkale ve yöresinde bulunan besi sığırlarında ayak hastalıklarının insidansının araştırılması amaçlanmıştır. Bu lokasyonda farklı ırk ve yaşlarda toplam 3047 adet büyükbaş besi sığırı ayak ve tırnak deformasyonları yönünden incelendi. Yılın belirli aylarında işletmeler ziyaret edilip ayak hastalıkları yönünden hayvanlar incelendi. Yapılan çalışmada hayvanların 53 adet hayvanın ayağında yayvan geniş tırnak, 1 adet hayvanda çift taban oluşumu, 1 adet hayvanda sivri tırnak, 1 adet hayvanda ayrık tırnak, 1 adet hayvanda makasvari tırnak, 1 adet hayvanda da çift taban oluşumu saptanmıştır. Ayak hastalığı olarak; 2 adet hayvanda interdigital dermatitis, 8 adet hayvanda digital dermatitis, 10 adet hayvanda ökçe çürüğü, 21 adet hayvanda taban ülseri, 5 adet hayvanda interdigital flegmon, 4 adet hayvanda beyaz çizgi hastalığı 22 adet hayvanın tırnağında ise laminitisin çeşitli formları tespit edildi. İşletmelerde ahır zemini, bakım koşulları ve yoğun besleme göz önüne alındığında, ahır hijyeni ve tırnak bakımının yetersiz olduğu belirlendi. Sonuç olarak ayak hastalıklarının oluşumunda ahır zemini ve rasyon içeriği gibi işletmelere ait yönetimsel faktörlerin etkisi, ırkın duyarlılık veya direncine göre farklılıkların olduğu belirlendi. Bütün bu faktörlerin eşliğinde istatiksel olarak elde edilen verilere göre Kırıkkale yöresindeki besi sığırlarının ayak hastalıklarının insidansı %4.23 olarak tespit edilmiştir.
Anahtar Kelimeler*: Ayak hastalıkları, Besi sığırı, Kırıkkale ili, İnsidans.*

Introduction

Closed and semi-closed farming system management practices are increasingly adopted in modern dairy and beef cattle farming to increase productivity. In closed barns, where high-yielding and heavy-bodied breeds are kept without access to pasture, hoof diseases are more commonly observed due to irregular wear resulting from insufficient hoof care (Şındak et al., 2003). Foot diseases constitute one of the most important health issues in cattle farming, causing significant financial losses in modern farms. Apart from the financial burden of treatment expenses for reforming and diseased animals, they lead to milk and productivity loss, extended calving intervals, reduced fertility in female animals, and additional costs associated with extra insemination. The etiology of foot diseases involves various factors, including external factors such as season, lactation in female animals, age, live weight, genetic predisposition, herd size, care conditions, nutrition, and farm-specific flooring structure (Dogra et al., 2020; Han et al., 2017; Jelinski et al., 2018; Keskin and Durmuş, 2016; Langova et al., 2020; Tutuş and Gençcelep, 2017; Yakan, 2018; Yayla et al., 2012; Yurdakul and Şen, 2018). Hoof disorders are frequently reported in dairy and beef cattle worldwide, accounting for approximately 90% of lameness cases. Hoof diseases can be classified as clinical or subclinical, and they are a source of pain and suffering for cattle due to the chronic and painful nature of the conditions (Abid et al., 1989; Kavuş, 2022; Tutuş and Gençcelep, 2017; Weaver, 1981). It has been reported that the incidence of hoof diseases is higher in intensive farming operations. Particularly in confined feedlot operations, factors such as limited exercise, accumulation of manure, and urine under animals triggered by the flooring structure play a significant role in the etiology of hoof diseases (Ishler et al., 2023). Depending on the environmental conditions, dermatitis, heel erosion, and secondary contamination agents such as Streptococcus, Staphylococcus, Spirochaete, and Bacillus species have been identified. Nutritional errors, such as excessively high carbohydrate content in the diet, can also lead to hoof deformities and laminitis (Berg and Loan, 1975; Cygan et al., 1977; Kamiloğlu, 2014; Masalski, 1986). As farms transition into more extensive operations with cattle trade, an increase in the incidence of infectious hoof diseases such as interdigital dermatitis, heel erosion, digital dermatitis, and interdigital phlegmon has been observed (Canpolat and Bulut, 2003; Özgen, 2014; Whitaker et al.,1983). The incidence of possible hoof diseases and lameness in herds has been reported to range from 0% to 60%. Especially subclinical hoof diseases often go unnoticed, leading to underestimated economic losses. Research shows that the problem of hoof diseases remains significant and is increasing (7-58%).

These rates have been reported as 0.3-3% in South Korea, 3.7% in Australia, 6.6% in the United States, 7.6% in Pakistan, 9.5% in Ireland, 10.9% in France, 16.4% in Switzerland, and 17.4% in the United Kingdom (Ablan, 1995; Murray et al., 1996; Özgen, 2014; Russell et al., 1982). Economic losses arise from hoof problems rather than treatment expenses. Depending on the severity of hoof diseases, they exhibit symptoms such as weight loss, decreased milk production and dry matter intake, reduced herd longevity, and fertility problems. Early diagnosis, prompt and regular treatment minimize losses, accelerate recovery, and prevent animal suffering (Anteplioğlu et al., 1992; Çeçen and Görgül, 2007; Greenough et al., 1981; Olcay and Sağlam, 1997; Sağlıyan and Ünsaldı, 2002; Şındak et al., 2003; Yavru et al., 1992). Digital dermatitis is a bacterial infectious disease affecting dairy cattle's feet. It is known to cause lameness, as well as a decrease in milk and reproductive performance. The current treatment protocol includes routine hoof trimming and topical antibiotics. Although several commercial topical products are used to control digital dermatitis lesions, there is limited or almost no evidence regarding their efficacy. Furthermore, it has been emphasized that washing the animals' feet with pressurized water at each milking session is more important than using non-antibiotic preparations compared to control groups where isotonic solutions were used. This approach can help prevent uncontrolled antibiotic use (McLennan, 1988; Özgen, 2014; Yücel, 1982).

Compared to dairy cattle farming, intensive beef cattle farming is more common in Kirikkale and its surrounding region. Due to the reported continuous financial losses experienced by farmers attempting to practice intensive beef cattle farming using traditional methods, this study aims to investigate and identify the possible causes of these losses. The study was conducted by examining a total of 3047 beef cattle from 6 different-scale farms in the Kirikkale region, representing different ages and breeds, to determine the incidence of existing hoof diseases and their causes.

Material and Methods

The study material consisted of a total of 3047 beef cattle from six different farms located in various regions of Kirikkale and its surrounding areas, where the population of beef cattle is high. The animals represented different ages, breeds, genders, and weights. It was determined that five of the examined barns had slatted concrete flooring (n=2445), while one barn had earthen flooring (n=602). Since the study did not involve an invasive intervention on animals, a letter was received from HADYEK (Animal Experiments Local Ethics Committee) that there was no need for ethics committee approval.

Anamnesis was obtained from the responsible farm veterinarian, farm workers, and farm owners during the farm visits. After examinations and observations, animals with hoof problems were identified. Animals with lameness complaints underwent detailed hoof examination to diagnose the diseases.

After completing the farm visits, the obtained data were evaluated to identify the relationships between disease types, disease-animal breed, disease-barn flooring, and disease-feeding management.

The adequacy of the housing conditions and the flooring structures of the six visited barns were examined. Anamnesis was obtained from the livestock owners regarding the frequency of hoof diseases and hoof deformities in cattle. The animals were examined for hoof diseases while standing and walking. The hoof and claw structures were examined for conformity with the animal's size, hoof deformities, abnormalities in the heel and sole regions, separations, shape changes, presence of fistulas, interdigital area diseases, and abnormalities in the corona region. All obtained data were recorded for further analysis. The records were evaluated using statistical methods, specifically the SPSS for Windows 20.0 Descriptive Statistics Analysis Crosstabs method, and the Chi-Square test was employed to investigate the effects of barn flooring types (concrete and earthen) on the occurrence of hoof diseases and hoof deformities in cattle. Based on the obtained results, significance levels of $P<0.05$ and $P<0.01$ were considered statistically significant for the study data.

Results

In this study, a total of 3047 beef cattle were examined for hoof diseases in the Kirikkale region. The examination results, together with the anamnesis obtained from the responsible farm supervisor, farm workers, and farm owners, were recorded in a patient follow-up form. As a result of this study, hoof lesions and foot diseases were detected in 100 animals out of the scanned 3047. It was found that some animals had lesions in one or more hooves, which prevented them from standing up.

Based on the research, it was observed that farms with a low number of animals and farms that did not employ any consulting services or veterinarians were not aware of hoof and claw care and diseases, and they were unaware of the magnitude of economic losses caused by these hoof and claw diseases. It was also observed that traditional methods were still being used to raise animals and that the hygiene and ventilation in the barns were inadequate.

In conscious farms, the presence of slatted concrete flooring in the barns was due to the aim of rapid weight gain through intensive feeding, which resulted in hoof diseases caused by imbalanced diets and sudden increases in the amount of barley in the diet. It was also observed that some farms had a pool for foot washing, which the animals passed through during monthly weight measurements, and it was observed that the farmers poured lime into this pool and used the vaccination route. In addition to nutrition, various premixes containing feed additives to prevent hoof diseases associated with intensive feeding, as well as zinc, copper, bicarbonate, and magnesium oxide, were observed.

The farm animals were mainly fed with clover at the beginning of the feeding period, as well as hay, corn silage, molasses, bran, crushed/cracked barley, corn meal, and commercial feed.

The visited farms raised animals of various breeds, including native breeds, European Angus breeds imported through private import companies, and animals imported from South American countries through the Meat and Milk Institution.

According to the obtained data, out of the scanned 3047 animals, hoof diseases were detected in 100 animals. In animals subjected to foot disease screening, some images of deformed hoof structures and foot diseases are presented in Figure 1 and Figure 2. Among these, the highest rate of hoof disease occurrence was found to be 18.36% in Holstein beef cattle, which are born in Turkey, while the breed with the lowest incidence of hoof diseases was determined to be Charolais, imported from European countries, with a rate of 1.33% (Table 1).

Table 1. Distribution of foot diseases among the researched animals in the Kirikkale region, categorized by breeds.

Animal Breeds	Examinated Animals	Number of Beef Cattle with Foot Disease	Ratio (%)
Native breed	293	16	5.46
S. American Angus	660	21	3.18
European Angus	488	9	1.84
Limousine	565	13	2.30
Charolais	672	9	1.33
Simmental	150	14	9.33
Holstein	49	9	18.36
Brangus	170	9	5.29
Total	3047	100	3.28

Table 2. The Rate of Occurrence of Foot Diseases in the Anteroposterior, Lateral-Medial Nails of the Beef Cattle Researched in the Kirikkale Region.

Localization of foot diseases	Lateral	Medial	Lateral+Medial	N
Front-Right	8	5	3	16
Front-Left	13	4	5	22
Behind-Right	11	15	2	28
Behind-Left	20	12	2	34
Total	**52**	**36**	**12**	**100**

Table 3. Distribution of Hoof Deformities Detected in Animals Investigated in the Kirikkale Region.

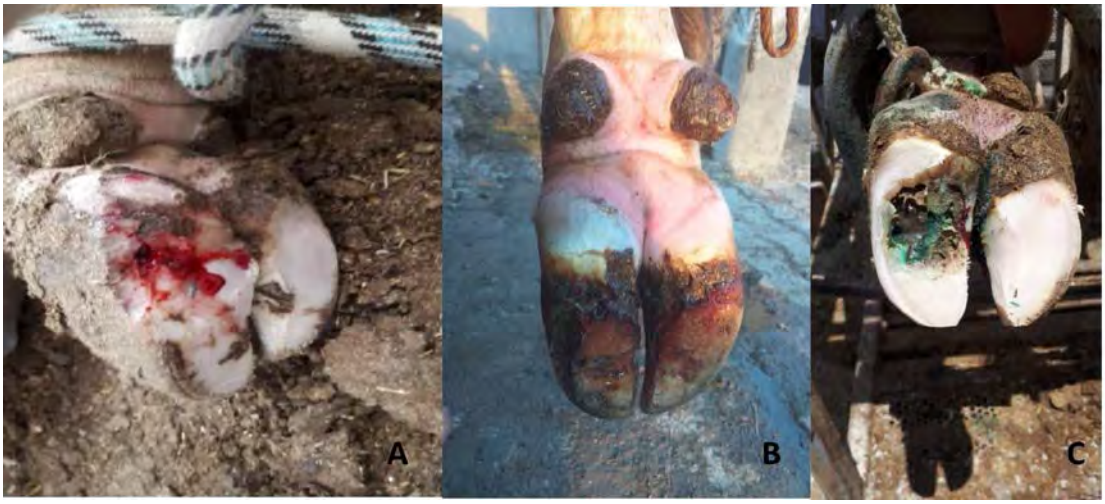
Hoof Deformation	N	Ratio(%)
Sprawled and Wide Hooves	53	91.37
Overgrown Hoof	1	1.72
Separated Hoof	1	1.72
Scissor-like Hoof	1	1.72
Double Sole Formation	1	1.72
Total	57	99,98

Table 4. Distribution of Foot Diseases Detected in Animals Investigated in the Kirikkale Region.

Foot Diseases	N	Ratio(%)
Interdigital Dermatitis	2	2,77
Digital Dermatitis	8	11,1
Hoof rot	10	13,88
Ulcus solea	21	29,16
Interdigital Phlegmon	5	6,94
White line disease	4	5,55
Laminitis	22	30,55
Total	**72**	**99,95**

A: Sprawled and wide hoof, **B:** Separated hoof and limax, **C:** Scissor-like hoof.

Figure 1. Some images of hoof deformities in animals investigated for foot diseases in Kirikkale region.



A: Solea abscess, **B:** Interdigital dermatitis and heel bruise, **C:** Ulcus solea.

Figure 2. Some images of foot diseases in animals investigated for foot diseases in Kirikkale region.

According to the examinations, when analyzing the distribution of diseases in the hooves, hoof diseases were detected in 30 hooves of the front limbs and 57 hooves of the hind limbs. The distribution of cases with hoof diseases in terms of front, rear, lateral, medial, and combined involvement is shown in (Table 2).

According to the data obtained from the study, a total of 57 animals (1.87%) were identified with hoof deformities (Table 3). It was determined that 2,445 % of the animals completed their feeding period in an open-semi-closed barn system with a concrete grid floor. The remaining 602 animals completed their feeding period freely in an open system with an earth floor.

According to the obtained data, out of the scanned 3047 animals, 72 animals (2.36%) were diagnosed with hoof diseases. The most common disease was laminitis, which was observed in 22 animals (30.5%), due to the intensive feeding system aiming for rapid weight gain in intensive beef cattle farming (Table 4).

Discussion and Conclusion

Increasing population growth has led to a rise in intensive cattle farming, where foot diseases are prevalent, especially in closed-confinement feedlots. Factors such as limited exercise opportunities due to confinement, the structure of the barn floor leading to the accumulation of manure and urine under the animals, and poor foot and barn hygiene, as well as feeding style and ration, play significant roles in the etiology of foot diseases (Han et al., 2017; Keskin and Durmuş, 2016; Yayla et al., 2012). In facilities with inadequate barn floors, cattle can experience lesions, laminitis, hemorrhages due to heel bruising, sole ulcers, white line separations, heel crush, and hoof cracks, which are considered triggering factors for foot diseases (Han et al., 2017; Vermunt and Greenough, 1995). The hardness and abrasive effect of concrete floors, in particular, lead to faster wear of the horn's hoof, resulting in lesions and lameness (Han et al., 2017; Vermunt and Greenough, 1995). Based on

the data obtained from the study, it was determined that the main issues in farms with high disease rates are the condition of the barn floor, inadequate hygiene, and insufficient preventive medicine. A secondary problem identified in the visited feedlots was the occurrence of subclinical and clinical laminitis cases due to ration errors.

Rapid live weight gain in feed animals, combined with flooring problems, results in a high incidence of sole lesions. In this study, farms with unsuitable barn floors, particularly those using grid-like concrete and hard surfaces, showed a high incidence of comprehensive claw deformities. Proper barn floor maintenance, hygiene, and adequate preventive measures are essential in such feedlots to prevent foot diseases and subsequent economic losses for the farmers.

The incidence of foot diseases was reported as 12.38% in a study conducted in the Tunceli region (Sağlıyan and Ünsaldı, 2002), and 17.1% in a study conducted in the Elazığ region (Canpolat and Bulut, 2003). A study conducted with dairy farms in the UK reported foot disease incidence ranging from 4.7% to 30% (Andersson and Lundstrom, 1981). In this current study, an incidence of 4.23% was found in feed cattle in the Kirikkale region. These findings indicate a significant presence of foot diseases in both beef and dairy cattle farming, leading to substantial economic losses that necessitate informing producers about the economic impact of foot diseases.

In cattle, the medial digits of the forelimbs and the lateral digits of the hind limbs carry more load. Consequently, foot diseases in cattle predominantly occur in the medial digits of the forelimbs and the lateral digits of the hind limbs (Anteplioğlu et al., 1992; Yavru et al., 1989). Our research in the Kirikkale region also revealed that foot diseases mainly occurred in the lateral digits of 21 animals and the medial digits of 9 animals in the forelimbs. In contrast, in the hind limbs, they occurred in the lateral digits of 31 animals and the medial digits of 26 animals.

Deformed hooves have been highlighted as important factors contributing to foot diseases (Ablan, 1995; Anteplioğlu et al., 1992; Görgül, 1983). According to some studies, more than 24% of hoof deformities have been reported in Turkey (Han et al., 2017; Kamiloğlu, 2014; Keskin and Durmuş, 2016; Yayla et al., 2012). Moreover, 90% of detected lameness cases were localized in the hooves in a study with 11,000 affected animals (Murray et al., 1996). Our study found 35 cases of foot diseases, 57 cases of hoof deformities, and 37 cases of both foot diseases and hoof deformities among 129 cattle in the Kirikkale region. These results indicate a high frequency of hoof deformities associated with foot diseases, emphasizing the importance of early diagnosis and treatment of hoof deformities.

Regarding the incidence of hoof deformities based on cattle breeds, our study identified 57 cases of deformed hooves among different cattle breeds in the Kirikkale region. Holstein cattle showed the highest incidence of foot diseases (18.36%), followed by South American Angus (Aberdeen Angus) (3.18%), and the native breed (5.46%). This suggests that specific cattle breeds, such as Holstein and South American Angus, are more susceptible to foot diseases, and it could be related to the system of the semi-open, closed concrete-gridded floors in feedlots.

In this study field, laminitis and sole ulcers were the most prevalent foot diseases, making up 30.55% and 29.16% of the identified cases, respectively. Based on the results, the majority of detected diseases were due to farm management reasons. Cattle with deformed hooves showed poor hoof maintenance, while cases of foot ulcers and subclinical laminitis required appropriate hoof care and medical treatment.

In conclusion, properly adjusting the ration according to the animals' needs, prioritizing barn hygiene, and regular hoof trimming in long-term feedlot operations are essential measures to reduce the incidence of foot diseases in feed cattle. The study highlighted the importance of educating farm managers and animal caregivers on these measures, which resulted in a reduction in foot diseases in farms with previously identified issues during the second visit.

Conflict of interest

The authors declare that there is no conflict of interest.

Ethical Approval

This study is not subject to HADYEK's permission in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees".

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References

Ablan L, 1995: Lameness in Danish dairy cows: frequency and possible risk factor. *Prev Vet Med,* 22, 213-225.

Abid TA, Eshoue SM, Badrany MS, Singh AP, 1989: Slaughter house survey of bovine foot disorders. *Indian Vet J,* 66:154-157.

Andersson L, Lundstrom K, 1981: The influence of breed, age, body weight and season on digital diseases and hoof size in dairy cow. *Zentralbl Veterinarmed A*, 28, 141-151.

Anteplioğlu H, Samsar E, Akın F, Güzel N, 1992: Sığır ayak hastalıkları (2.baskı) Ankara Üniversitesi Veteriner Fakültesi Yayınları, Ankara.

Berg JN, Loan RW, 1975: Fusobacterium necrophorum and bacteroides melaninogenicus as etiologic agents of foot rot in cattle. *Am J Vet Res,* 36 (08), 115-1122.

Canpolat İ, Bulut S, 2003: Elazığ ve çevresinde sığırlarda görülen ayak hastalıklarının insidansı üzerine gözlemler. *Fırat Univ Sag Bil Derg,* 17, 155-160.

Cygan Z, Wiercinsky J, Szewczuk P, 1977: Experimental study on the aetiology of interdigital necrobacillosis in cattle. *Med Weter*, 33, 720-724.

Dogra S, Singh R, Ravinder S, Tikoo A, 2019: Effect of claw disorders on haemato-biochemical parameters and acute phase protein levels in crossbred cattle. *Indian J Anim Res*, 54 (2), 173-176.

Görgül O, 1983: Sığırların önemli ayak hastalıklarında operatif sagaltım yöntemleri. *Uludağ Univ Vet Fak Derg,* 2, 21-29.

Greenough PR, MacCallum FJ, Weaver AD, 1981: Lameness in Cattle. 2nd ed., Wright Scientechnica, Bristol, UK.

Han MC, Sağlıyan A, Polat E, 2017: Sığırlarda ahır zemin tiplerinin ayak hastalıkları ve tırnak deformasyonları üzerine etkilerinin araştırılması. *Harran Üniv Vet Fak Derg,* 6 (1), 19-24.

Ishler V, Wolfgang D, Griswold D, 2023: Prevention and control of foot problems in dairy cows. https://dairy-cattle.extension.org/prevention-and-control-of-foot-problems-in-dairy-cows/, Date of Access; 15.05.2023.

Jelinski M, Waldner C, Penner G, 2018: Case-control study of mineral concentrations of hoof horn tissue derived from feedlot cattle with toe tip necrosis syndrome (toe necrosis). *Can Vet J*, 59, 254.

Kamiloğlu A, 2014: Çiftlik hayvanlarında ayak hastalıkları. Medipress, Malatya.

Kavuş MT, 2022: Elazığ ve Erzincan Yöresi Sığır Ayak Hastalıklarının Değerlendirilmesi. Yüksek Lisans Tezi, Fırat Üniversitesi Sağlık Bilimleri Enstitüsü, Elazığ.

Keskin E, Durmuş AS, 2016: Gaziantep ve yöresinde gözlenen sığır ayak hastalıklarının insidansı ve tedavileri üzerine gözlemler. *SÜ Sağ Bil Vet Derg,* 30 (3), 181-86.

Langova L, Novotna I, Nemcova P, Machacek M, Havlicek Z, Zemanova M, Chrast V, 2020: Impact of Nutrients on the Hoof Health in Cattle. Animals. 10(10):1824. https://doi.org/10.3390/ani10101824.

Masalski N, 1986: Properties of Bacteroides nodosus isolated from cattle. *Veterinarnomeditsinski Nauki,* 23, 32-36.

McLennan MW, 1988: İncidence of lameness requiring veterinary treatment in dairycattle in Queensland. *Aust Vet J*, 65, 144-147.

Murray RD, Downham DY, Clarkson MJ, Faull WB, Hughes JW, Manson FJ, Merritt JB, Russell WB, Sutherst JE, Ward WR, 1996: Epidemiology of lameness in dairy cattle: description and analysis of foot lesion. *Vet Rec,* 138 (24), 586-591.

Olcay B, Sağlam M, 1997: Büyük Hayvanların Ayak Hastalıkları ve Ortopedisi. Ankara Üniversitesi Veteriner Fakültesi Ders Notları, Ankara.

Özgen E, 2014: Erzurum Yöresi Koyunlarında Görülen Ayak Hastalıklarının Prevalansı. Yüksek Lisans Tezi, Kırıkkale Üniversitesi Sağlık Bilimleri Enstitüsü, Kırıkkale.

Russell AM, Rowlands GJ, Shaw SR, Weaver AD, 1982: Survey of Lameness in british dairy cattle. *Vet Rec,* 111, 155-160.

Sağlıyan A, Ünsaldı E, 2002: Tunceli ve yöresindeki sığırlarda görülen ayak hastalıklarının prevalansı üzerine gözlemler. *Fırat Univ Sag Bil Derg*, 16, 47-56.

Şındak N, Keskin O, Selçukbiricik, H, Sertkaya H, 2003: Şanlıurfa ve Yöresinde Sığır Ayak Hastalıklarının Prevalansı. *YYÜ. Vet Fak Derg,* 14 (1), 14-18.

Tutuş D, Gençcelep M, 2021: Van Muradiye İlçesinde Ruminantlarda Görülen Ekstremite ve Ayak Hastalıklarının İnsidansı. *Van Vet J,* 32 (2), 82-90.

Vermunt JJ, Greenough PR, 1995: Structural characteristics of the bovine claw: Horn growth and wear, horn hardness and claw conformation. *Br Vet J,* 151, 157-180.

Yakan S, (2018). Ağrı İlinde Sığırlarda Ayak Hastalıkları Prevalansının Belirlenmesi. *Harran Univ Vet Fak Derg,* 7(2), 207-212.

Yavru N, Özkan K, Elma E, 1994: Ayak hastalıkları ve ortopedisi, Selçuk Üniversitesi Veteriner Fakültesi basım ofset matbaası, Konya.

Yayla S, Aksoy Ö, Kılıç E, Cihan M, Özaydın İ, Ermutlu CŞ, 2012: Kars ve yöresinde sığırların bakım ve barındırma koşulları ile ayak hastalıkları arasındaki ilişkinin değerlendirilmesi. *Harran Univ Vet Fak Derg,* 1(1), 22-27.

Yurdakul İ, Şen İ, 2018: Sivas ve yöresinde sığır ayak hastalıkları prevalansının belirlenmesi. *Harran Univ Vet Fak Derg,* 7(1), 51-55.

Yücel M, 1982: İstanbul ve Tekirdağ bölgesindeki sığırlarda görülen ayak hastalıklarının toplu bir değerlendirilmesi. *İstanbul UnivVet Fak Derg,* 8, 47-61.

Weaver AD, 1981: Lameness in Cattle, Wright Scientechnica, 1nd Ed. Bristol.

HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Investigation of Histological and Histochemical Features of Mouse Placenta in Different Periods of Pregnancy*

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Abstract: This study aimed to investigate the histological and histochemical properties of mouse placenta samples from different periods of pregnancy. For this purpose, mouse placenta samples were collected on the fourth, tenth, and seventeenth days of pregnancy and blocked in paraffin. Serial sections of 5µ thickness were taken at 50µ intervals. Histological and histochemical staining methods were applied to the sections. As a result, histological and histochemical characteristics of mouse placenta from different periods of pregnancy were determined.

Keywords: *Histochemistry, Histology, Mouse, Placenta.*

Gebeliğin Farklı Dönemlerindeki Fare Plasentasının Histolojik ve Histokimyasal Özelliklerinin İncelenmesi

Özet: Bu çalışmada gebeliğin farklı dönemlerine ait fare plasenta örneklerinin histolojik ve histokimyasal özelliklerinin araştırılması amaçlanmıştır. Bu amaçla gebeliğin dördüncü, onuncu ve on yedinci günlerindeki fare plasenta örnekleri toplanarak parafinde bloklandılar. 50µ ara ile 5µ kalınlığında seri kesitler alındı. Kesitlere histolojik ve histokimyasal boyama yöntemleri uygulandı. Sonuç olarak gebeliğin farklı dönemlerine ait fare plasentasının histolojik ve histokimyasal özellikleri belirlendi.

Anahtar kelimeler: *Fare, Histokimya, Histoloji, Plasenta.*

Introduction

Placenta; it is an extraembryonic tissue that creates an environment for the fetus to develop in the uterus and is found only in mammals. The placenta, which carries out the feto-maternal exchange of nutrients and substances during pregnancy, also serves as a comprehensive endocrine organ (Furukawa et al., 2011; Tewari et al., 2011).

Since developmental and experimental studies on the human placenta are not ethically possible, animal placentas are used as placenta models. The Mouse placenta is preferred as an animal model in many studies due to its phylogenetically similar structure to humans, ease of supply, suitability of pregnancy and cycle duration, and ease of animal care (Adams et al., 2020; Wu et al., 2018; Yu et al., 2018; Zhu et al., 2019).

The mouse placenta is a chorioallantoic type placenta and has a discoidal shape. Histologically, it is classified as hemotrichorial (Furukawa et al., 2011). It is also classified as hemoendothelial in some sources (Özer, 2007). The mature mouse placenta is fully formed in 10.5 days. It is shaped from three layers; 1. Labyrinth zone 2. Connection zone 3. Maternal decidua. It also contains a special membrane called Reichert's Membrane, which is unique to rodents (Chavatte-Palmer and Tarrade, 2016; Furukawa et al., 2014; Hafez, 2017; Johnson and Everitt, 2018; McGeady et al., 2017; Rai and Cross, 2014; Sinowatz, 2009).

This study aimed to investigate mouse placenta samples' histological and histochemical properties from the fourth, tenth, and seventeenth days of pregnancy.

Material and Methods

In the present study, 21 female and 7 male mice of the CD-1 race, aged 8-10 weeks were used. The vaginal plug was checked every morning and the pregnant ones were placed in separate cages. Those with a positive vaginal plug were considered to be on the first day of pregnancy. Seven healthy mouse placentas were obtained as material from animals euthanized by cervical dislocation under ether anesthesia on the fourth, tenth, and seventeenth days of pregnancy. After the tissue materials were fixed in 10% neutral buffered formaldehyde for 24 hours, they went through routine tissue tracking stages and were embedded in paraffin. Serial sections of 5µ thickness were taken from the prepared paraffin blocks at 50µ intervals (Koç and Kum, 2022). Histological and histochemical staining methods (Table 1) were applied to the sections obtained from paraffin blocks. The obtained data were evaluated light microscopically (Leica DMLB, Made in Germany). For this study, permission was received from Aydın Adnan Menderes University Animal Experiments Local Ethics Committee (ADU HADYEK 64583101/2016/017).

Table 1. Applied staining methods and purposes of the methods.

METHOD	PURPOSE OF THE METHOD
Hematoksilen-Eozin (Culling et al, 1985)	Determination of general histological appearance
Triple Stain (Culling et al, 1985)	Determination of general histological appearance
Periyodik Asit-Schiff(PAS) (Culling et al, 1985)	Demonstration of neutral mucosubstance
Alsian Blue pH 2.5 (AB pH 2.5)(Culling et al, 1985)	Determination of carboxylated acidic mucosubstance
PAS/AB pH 2.5 (Culling et al, 1985)	Coexistence of neutral and acidic mucosubstance
Aldehit Fuksin (AF) (Culling et al, 1985)	Determination of sulfated acidic mucosubstance
AB pH 1.0 (Culling et al, 1985)	Determination of O-sulfate ester mucosubstance
AB pH 0.5 (Culling et al, 1985)	Determination of strong sulfated mucosubstance
AF/AB pH 2.5 (Culling et al, 1985)	Coexistence of sulfated and carboxylated acidic mucosubstance

Results

Fourth day: On the fourth day, it was observed that the histological structure of the uterus was preserved. The structure of the endometrium, myometrium, and perimetrium was generally normal (Figure 1A). No implantation was observed on the fourth day. It was observed that the structure and integrity of the epithelial cells in the endometrium lamina epithelium began to deteriorate in some places. It was observed that stromal cells were changed in the lamina propria (Figure 1B). It was observed that the cells that started to change often had more than one nucleolus. Cells containing vacuolar structures were found in the lamina propria and between epithelial cells. It was observed that the glands became small, and the structure of the gland epithelial cells and the stroma around the glands changed. A large number of neutrophils were detected in the functional lamina propria of the endometrium. Again, in most samples, numerous capillary blood vessels were observed in the functional endometrium. Vascular structures arranged in a clear line were also seen at the end of the endometrium basalis.

The reactions observed with histochemical staining methods applied on the fourth day of pregnancy are given in Table 2.

Moderate PAS positivity was observed on the surface of the lamina epithelialis, basement membrane, and gland epithelial cells. At Alcian blue pH 2.5, a weak reaction was observed on the lamina epithelialis surface, gland epithelial surface, and serosa. PAS/AB pH 2.5 staining determined that the AB reaction was dominant on the lamina epithelialis and gland epithelial surface. AF staining revealed a strong,

sometimes moderate, and low-grade AF positive reaction on the epithelial surface (Figure 2A). A moderate reaction was observed on the surface of the gland epithelial cells (Figure 2B). A strong AF reaction was detected in the serosa (Figure 2C) and the vessel wall (Figure 2D). A moderate reaction was observed on the gland epithelial surface in AB pH 1.0 and 0.5 methods (Figure 3A). A strong reaction was detected in the serosa (Figure 3B). In the AF/AB pH 2.5 staining method, it was noted that the AB reaction was dominant on the surface of the lamina epithelialis and gland epithelium. In the serosa, AF and AB reactions were observed to be similar.

Table 2. Histochemical reactions on the fourth day of pregnancy.

	PAS	AB pH 2.5	PAS+AB	AF	AB pH 1.0	AB pH 0.5	AF+AB
Lamina epithelialis	(++)	(+)	AB	(++)	(+)	(++)	Mostly AB, some parts AF
Gland epithelium	(++)	(+)	-	(++)	(++)	(+)	AB
Stroma	(++)	-	-	(+++)	-	-	-
Vascular Wall	(++)	-	-	(+++)	-	-	-
Serosa	(++)	(+)	PAS	(+++)	(+++)	(+++)	AF, AB, AF/AB

None (-), Weak (+), Medium (++), Strong (+++).

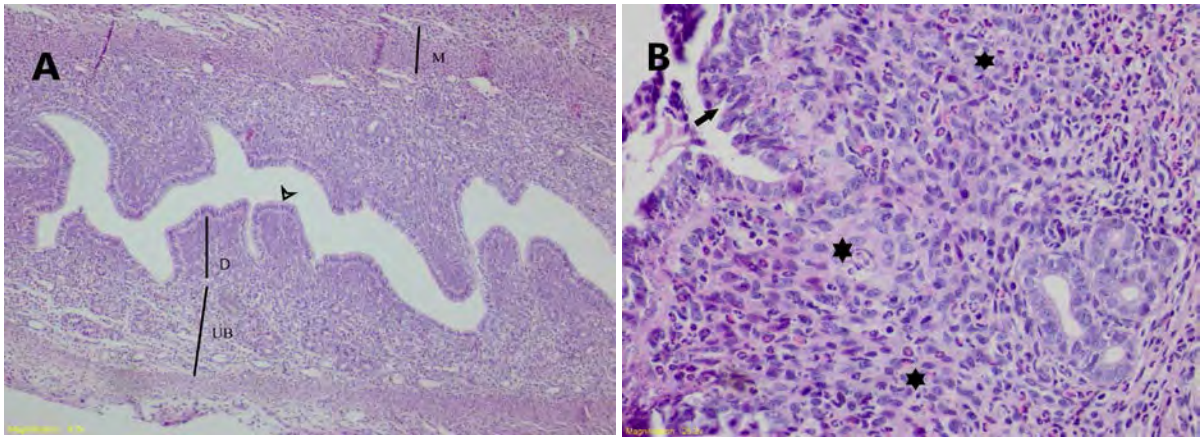

Figure 1. The fourth day of pregnancy. **A.** Lamina epithelialis (arrowhead), lamina propria part where cellular changes are seen (D), lamina propria where uterine glands are concentrated (UB), and myometrium (M). Hematoxylin-Eosin staining method. Bar 200 µm. **B.** Deterioration in epithelial integrity (arrow), changes in stroma cells (*). Hematoxylin-Eosin staining method. Bar 50 µm.

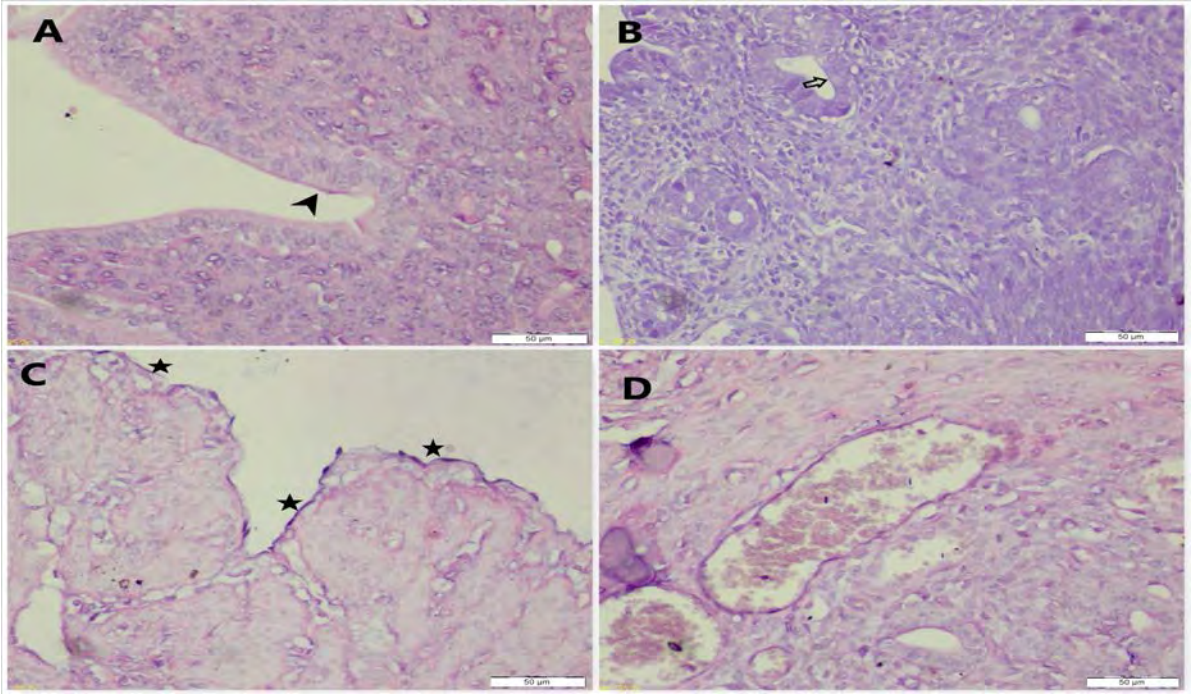

Figure 2. The fourth day of pregnancy. A) AF (+) reaction (arrowhead) on the lamina epithelialis surface. B) AF reaction observed on the surface of glandular epithelial cells (open arrow) C) AF (+) reaction on the serosal surface (*). D) AF (+) reaction in the vessel wall. Aldehyde Fuchsin staining method. Bar 50 µm.

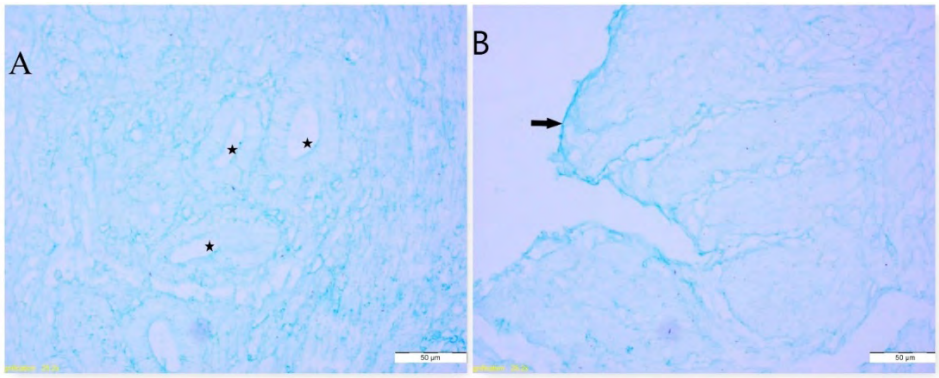

Figure 3. The fourth day of pregnancy. A) Moderate AB reaction on the glandular epithelial surface (*). B) Strong AB reaction (arrow) in the tunica serosa. AB pH 1.0 staining method. Bar 50 µm.

Tenth day: On the tenth day, fetal and maternal structures could be clearly distinguished. The mesometrial and antimesometrial parts of the uterus were distinguished (Figure 4A). It was observed that the amniotic cavity grew noticeably and the amniotic membrane surrounded the fetus. It was observed that parietal trophoblastic giant cells (P-TGCs) surrounded the border of the entire amniotic cavity under the amniotic membrane. It was observed that the antimesometrium was much thinner than the mesometrium. In this section, as you move from the amniotic cavity towards the serosa; At first, single or several rows of trophoblastic giant cells (TGCs) were found in the amniotic membrane and the layer just below it (Figure 4B). Laterally, it was observed that P-TGCs began to separate into islets, their size increased compared to the antimesometrium, and blood pools began to be seen here and there between the islets. It was noted that there was a dense layer of antimesometrial decidua layer (decidua parietal) just below the P-TGCs (Figure 4B). The striking feature of antimesometrial decidua was the lack of vascularization. It was observed that vascularization increased towards the lateral and mesometrium. It was observed that the endometrial epithelium and its lumen were pushed between the antimesometrial decidua layer and the myometrium, and the lumen narrowed considerably (Figure 4B). It was observed that all these structures were surrounded by myometrium and perimetrium, respectively.

It was determined that the density of the decidua layer decreased and vascularization increased in the mesometrial section. It was observed that gaps began to form laterally between the decidua cells (Figure 5A). Structures belonging to the labyrinth of the placenta began to be seen under the amniotic membrane surrounding the amniotic cavity in the mesometrium (Figure 5B). Trophoblastic giant cells and syncytiotrophoblast (SynT) cell clusters were scattered. Maternal blood elements and fetal blood vessels were seen among these clusters. Fetal and maternal blood could be easily distinguished because fetal erythrocytes were darker stained and nucleated (Figure 5C, 5D). It was observed that there were several arranged and scattered trophoblast clusters around the fetal blood vessels. Maternal blood cells were located in irregular spaces in many parts of the labyrinth (Figure 5C, 5D).

Several cell layers, presumably a syncytiotrophoblast layer, were observed where the labyrinth zone ends. Following this layer, the P-TGC cell row was followed in a single or two rows. Laterally, P-TGCs were determined to be multi-row (Figure 6A, 6B). The main channel structure was observed just at the end of the labyrinth layer (Figure 6C). It was determined that the size of P-TGCs in the mesometrium was reduced compared to the antimesometrial and lateral ones. Following the P-TGCs sequence, a mesometrial decidua layer was observed up to the myometrium (Figure 6C, 6D). It was observed that spongiotrophoblast cells (SpTC) and glycogen cells (Gly-C) began to form in the decidua basalis just below the P-TGCs, and blood pools surrounded the cell groups. It was determined that SpTC was located in several rows just below the P-TGCs and the connection zone began to form here (Figure 6C, 6D, 6E, 6F). When examined from the connection zone towards the serosa, it was determined that decidua and Gly-C were fused and were not clearly distinguished. In the mesometrium, it was observed that decidua concentrated and Gly-C decreased. In a part close to the mesometrium, lymphocyte infiltrations were seen among the decidual tissue in the area called MLAp (Mesometrial lymphocyte community during pregnancy), separated by the myometrium (Figure 6G, 6H).

The reactions observed with histochemical staining methods applied on the tenth day of pregnancy are given in Table 3.

It was observed that the antimesometrium decidua cell borders became apparent with a medium-strength reaction with PAS (Figure 7A). While a medium-strength reaction was observed at the cell borders and the parts bordering the cell islets in the decidua basalis of the mesometrium, an extreme reaction was observed in the glycogen cells (Figures 7B, 7D). A granular PAS reaction was noted in the cytoplasm of glycogen cells (Figure 7C, 7D). In the AB pH 2.5 staining method, a weak reaction was observed in the uterine lumen in the antimesometrium, while a moderate reaction was observed in the serosa and lateral P-TGC. In the PAS/AB pH2.5 staining method, a large amount of PAS positivity was detected on the lamina epithelial surface in anti-mesometrium (Figure 8A). Although PAS positivity was mostly found in the serosa, reactions of similar intensity were observed (Figure 8B). PAS positivity was observed in

the junction zone glycogen cells and MLAp cells of the mesometrium (Figure 8C). In the labyrinth, PAS positivity was observed on the surface adjacent to the amniotic cavity, while AB positivity was observed in the stromal areas within the labyrinth (Figure 8D). In the aldehyde fuchsin staining method, reactions of different intensities were observed in the labyrinth, P-TGCs, and serosa. In the AB pH 1.0 staining method, a strong positive reaction was observed in the serosa, and a moderate positive reaction in the decidua (Figure 9). The weak reaction was seen in glycogen cells and the labyrinth.

In the AB pH 0.5 staining method, a moderate reaction while in the labyrinth and a weak reaction in the glycogen cells of the decidua basalis was detected. In the AF/AB pH 2.5 staining method, AF positivity was detected in the antimesometrium and mesometrial serosa.

Table 3. Histochemical reactions on the tenth day of pregnancy,

	PAS	AB pH2.5	PAS+AB	AF	AB pH 1.0	AB pH 0.5	AF+AB
Antimesometrial Decidua	(++)	(Lamina epithelialis +)	PAS, PAS/AB	(++)	-	(++)	AF, AF/AB
Antimesometrial P-TGC	(+++) Granular	(++)	PAS	(++)	-	-	AF
Mesometrial P-TGC	(+++)	(++)	PAS	Reaction on the cell surface	(+)	(++)	AF
Labyrinth	(++)	(++)	AB	(++)	(+)	(++)	AF
Mesometriaal Decidua	(++)	(++)	PAS	(++)	(++)	(+)	AF
Glycogen Cells	(+++) Granular	(++)	PAS	(++)	(+)	(++)	AF
MLAp	(+++)	(+)	PAS	(++)	(++)	(+)	AF
Serosa	(++)	(+)	PAS, PAS/AB	(++)	(++)	(++)	AF, AF/AB

None (-), Weak (+), Medium (++), Strong (+++).

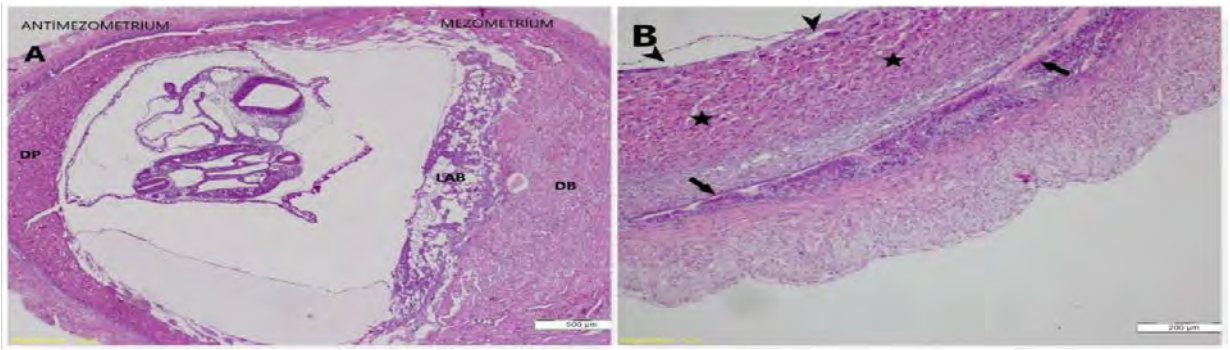

Figure 4. Tenth day of pregnancy. **A.** DP: Decidua parietalis, DB: Decidua basalis, LAB: Labyrinth. Bar 500 µm. **B.** Antimesometrial part of the uterus (decidua pariyetalis). P-TGC (arrowheads), decidua parietalis surrounding the amniotic cavity (*), endometrium lamina epithelialis (arrows). Hematoxylin-Eosin staining method**.** Bar 200 µm.

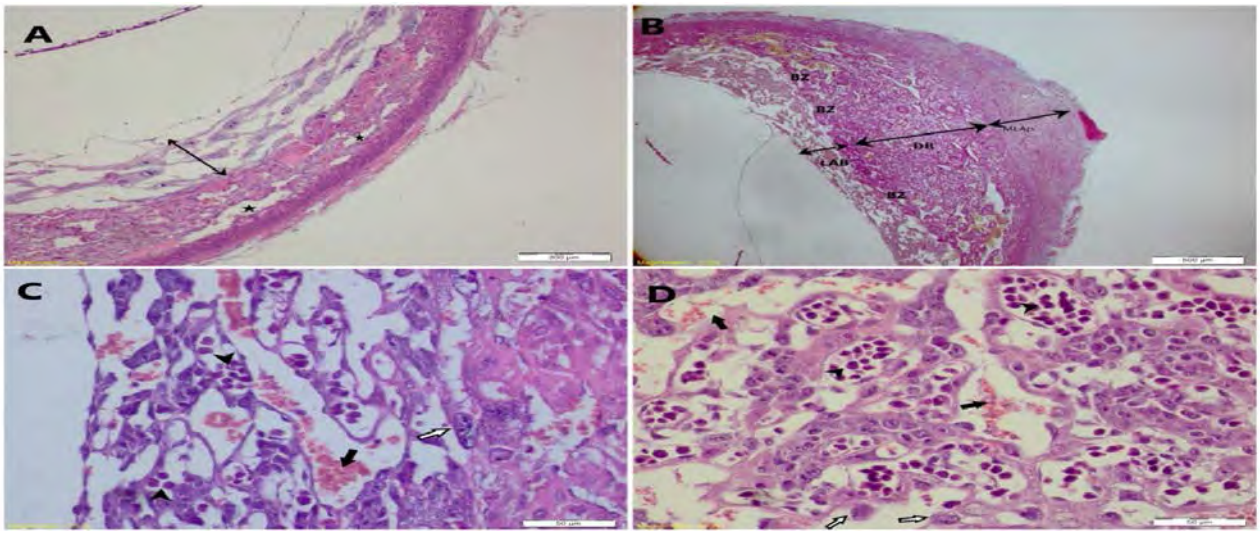

Figure 5. Tenth day of pregnancy. **A.** Lateral decidua areas. P-TGCs (double-sided arrow) and detachments start to form in the decidua (*). Bar 200 µm. **B.** Mesometrial part. LAB: Labyrinth, BZ: Connection zone, DB: Decidua basalis, MLAp. Triple staining method. Bar 500 µm. **C, D.** The labyrinth part in development. Nucleated fetal erythrocytes (arrowhead), maternal blood spaces (black arrow), TGCs (white arrow). Hematoxylin-Eosin staining method. Bar 50 µm.

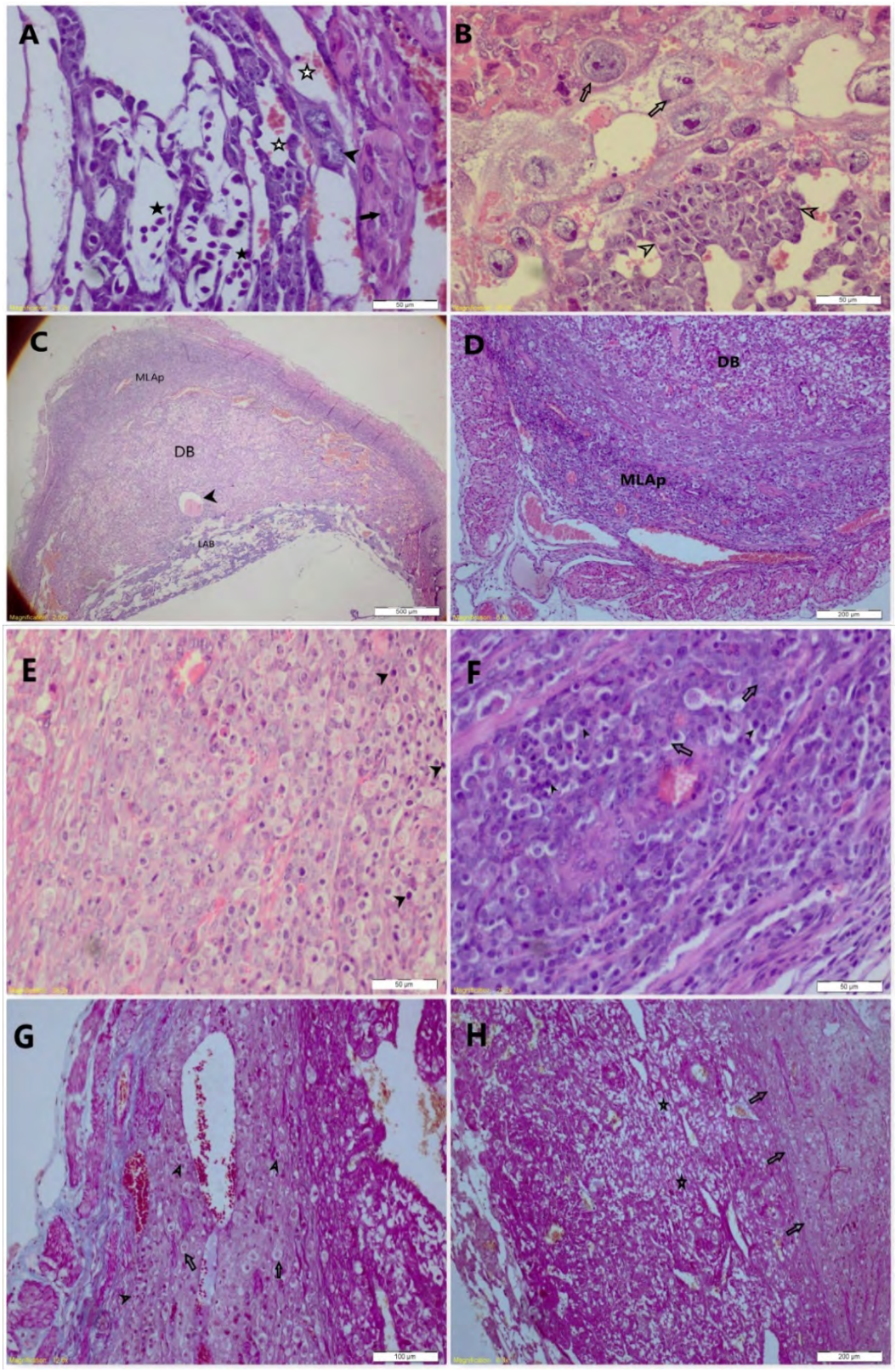

Figure 6. Tenth day of pregnancy**.**
A. Labyrinth-connection zone transition line. Erythrocytes with darkly stained nuclei (black stars) within fetal blood vessels. Nucleated erythrocytes (white stars) within maternal blood spaces. P-TGCs (arrowheads) bordering the labyrinth. Spongiotrophoblasts at the beginning of the junction zone. Hematoxylin-Eosin staining method. Bar 50 µm. **B**. Labyrinth-connection zone transition line. P-TGCs (white arrow), Syncytiotrophoblasts (white arrowhead). Bar 50 µm. **C**. Mesometrium. LAB: Labyrinth, DB: decidua basalis, MLAp, main channel structure that brings blood to the labyrinth (arrowhead). Hematoxylin-Eosin staining method. Bar 500 µm. **D**. MLap and DB (Decidua basalis) image. Bar 500 µm. **E-F**. MLap (arrowheads), decidua (arrows). Hematoxylin-Eosin staining method. Bar 50 µm. **G**. MLap. Decidua (white arrows), lymphocyte infiltrates (arrowheads). Triple staining method. Bar 500 µm. **H.** Glycogen cells in decidua basalis (stars), end of decidua basalis, beginning of MLAp (arrows). Hematoxylin-Eosin staining method. Bar 200 µm.

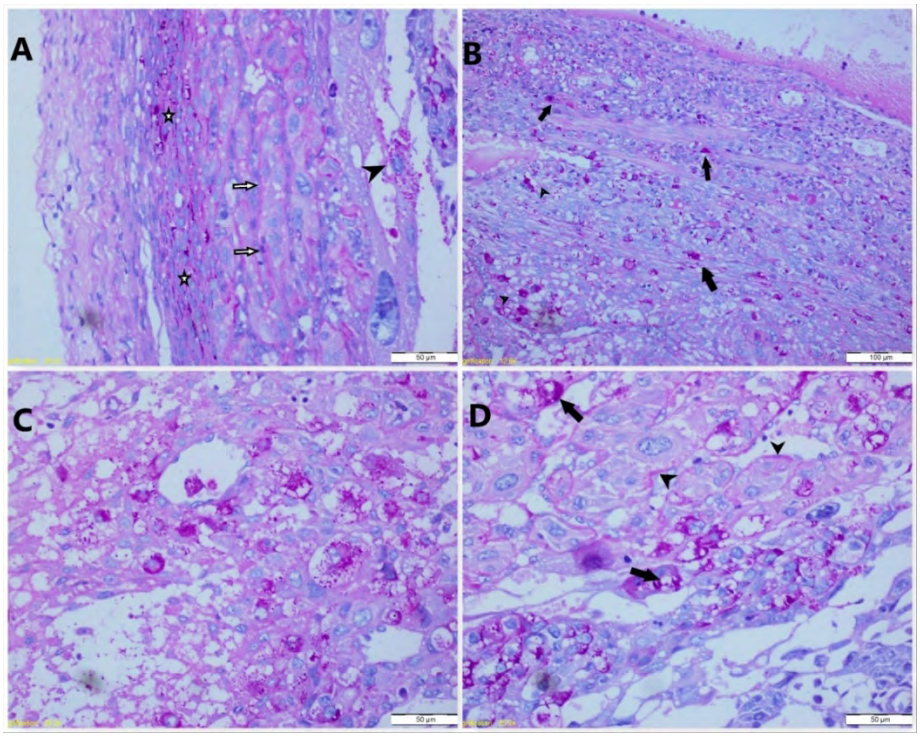

Figure 7. Tenth day of pregnancy**.** A) Decidua cells (white stars) showing strong PAS positivity in the antimesometrium, cell borders with decidua groups showing moderate reaction (white arrows), and P-TGC (black arrowhead) showing strong reaction in a granular manner. Bar 50 µm. B) Mesometrium. MLap and decidua basalis transition zone. Glycogen cells with strong cytoplasmic reactions (black arrowheads). Cells giving PAS positivity within MLAp, are thought to be uNK cells (black arrows). Bar 100 µm. C) Glycogen cells showing strong cytoplasmic reaction. Bar 50 µm. D) Labyrinth-connection zone-decidua basalis transition zone. Strongly reacting glycogen cells (arrowsBorders of decidua cell groups (arrowheads) showing moderate strength reaction. PAS staining method. Bar 50 µm.

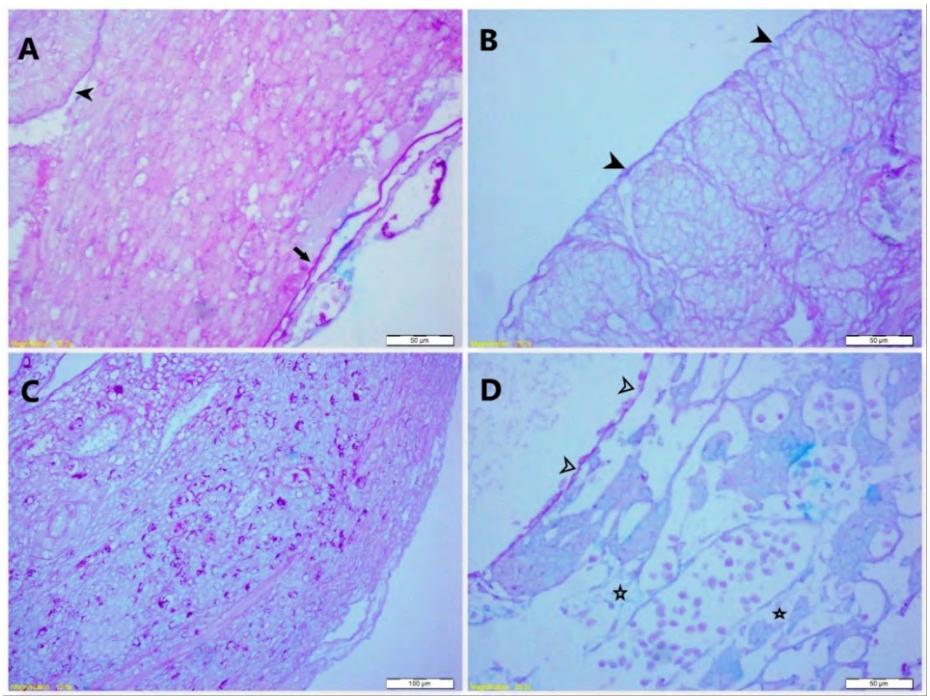

Figure 8. Tenth day of pregnancy. A) PAS (+) and AB (+) reaction (arrowhead) in the lamina epithelium of the antimesometrium and PAS positivity (arrow) at the border of the amniotic cavity. Bar 50 µm. B) PAS (+) and AB (+) reaction in the serosa (arrowheads). Bar 50 µm. C) General view of the decidua basalis-MLAP transition zone. Bar 100 µm. D) Labyrinth. Amniotic cavity surface showing predominant PAS positivity (white arrowheads). Labyrinth areas (white stars) showing AB positivity. PAS-AB pH 2.5 staining method. Bar 50 µm.

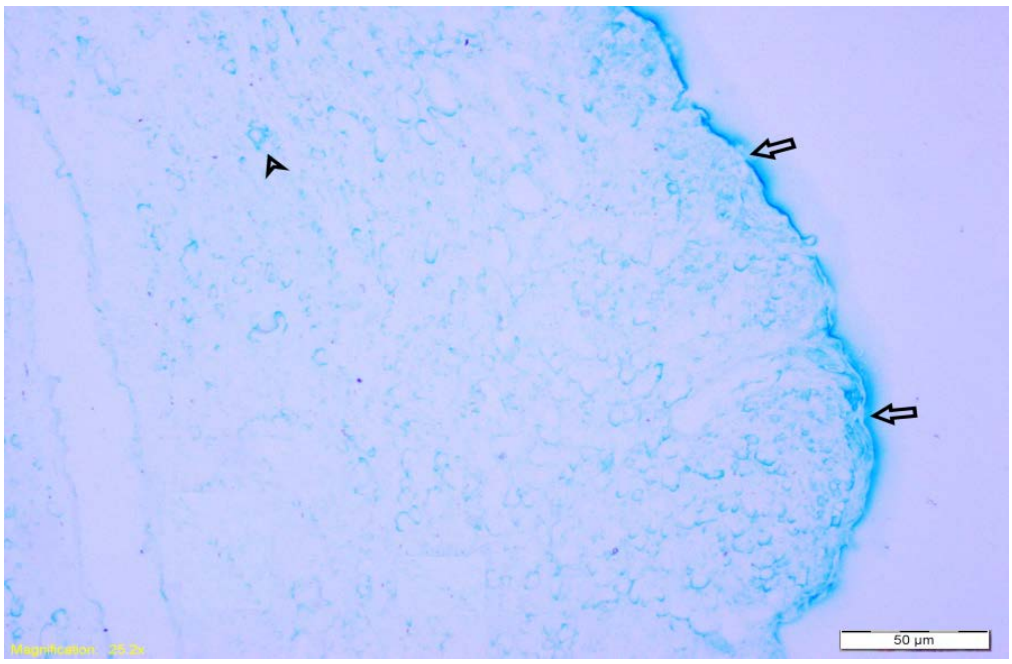

Figure 9. Tenth day of pregnancy. Strong AB pH 1.0 reaction in the serosa (arrows) and moderate reaction in the decidua (arrowhead). AB pH 1.0 staining method. Bar 50 µm.

Seventeenth Day: On the seventeenth day, a mature mouse placenta structure was observed. It was observed that the placenta consisted of four different main parts: decidua basalis, connection zone, labyrinth, and chorionic plate (Figure 10A, 10B). It was determined that decidua basalis extends from the mesometrium to the P-TGCs cells. It was noted that the presence of MLAp continued in the parts close to the mesometrium. Lymphocyte infiltrates were observed in this area (Figure 11A, 11B). Darkly stained spongiotrophoblast cells were detected in the junction zone and glycogen cells with light-colored foamy cytoplasm were detected between these cells (Figure 12A). At the border of the labyrinth, spongiotrophoblast cells were observed to make indentations in the labyrinth in the form of villus-like structures. In the part where the connection zone meets the decidua basalis, generally, single-row P-TGC cells bordering the basal zone were noted (Figure 12B, 12C, 12D). At the end of the connection zone, the presence of another fetal part, the labyrinth, was observed. In the labyrinth, there are generally fetal blood elements (Figure 13A, 13B), fetal endothelium, syncytiotrophoblasts (SynTC), and around them sinusoid trophoblastic giant cells (S-TGC) (Figure 13D, 13F), maternal blood pools and maternal erythrocytes circulating freely in these pools. was seen. Syncytiotrophoblasts were not clearly observed in all sections. The presence of a large channel was seen inside the labyrinth (Figure 13C, 13E). The presence of maternal erythrocytes was detected in this channel (Figure 13E). It was observed that channel trophoblastic giant cells (C-TGC) were lined up around this channel (Figure 13C, 13E). At the end of the labyrinth section, the chorionic plate section, facing the amniotic cavity, with dense vascular and channel structures in the center of the placenta, was seen (Figure 13F). In this section, channel trophoblastic giant cells and S-TGCs were observed. Reichert's Membrane, which borders the placenta and extends as a thin line under the amnion and yolk sac layer that surrounds the entire placenta from the embryonal side, was noted (Figure 13H). This membrane was observed to be a homogeneous and avascular layer in all parts. The presence of a developed yolk sac with a basement membrane and an epithelium, spreading towards the amniotic cavity outside the placenta, was noted (Figure 13G, 13H).

The reactions observed with histochemical staining methods applied on the seventeenth day of pregnancy are given in Table 4.

On the seventeenth day, strong PAS positivity was observed on the basement membrane and epithelial surface of the yolk sac (Figure 14A). A very strong granular reaction was observed in the glycogen cells within the junction zone (Figure 14B). PAS-positive cells, thought to be uNK cells, were found in the MLAp-compatible region of the decidua basalis (Figure 14C, 14D). In the AB pH 2.5 staining method, moderate positivity was observed on the basement membrane of the yolk sac, and weak positivity was observed on the epithelial surface. In the PAS/AB pH 2.5 staining method, PAS was dominant on the yolk membrane and epithelial surface. In the AF staining method, medium strength positivity was observed on the yolk sac epithelial surface, vascular endothelium in the labyrinth, and vascular walls in the chorionic plate. In the AB pH 1.0 staining method, different strengths of reaction were observed in the serosa and glycogen cells. In the AB pH 0.5 staining method, a moderate reaction was detected on the serosa and yolk sac epithelial surface. In the AF/AB pH 2.5 staining method, AF positivity was observed on the basement membrane and epithelial surface of the yolk sac.

Table 4. Histochemical reactions on the seventeenth day of pregnancy.

	PAS	AB pH 2.5	PAS+AB	AF	AB pH 1.0	AB pH 0.5	AF+AB
Mesometrial decidua (Decidua basalis)	(+++)	-	PAS	(+++)	-	(++)	AF
Connection zone Spongiotrophoblast	(+++)	-	PAS	(++)	-	-	AF
Connection zone Glycogen Cells	(+++)	(+)	PAS	(+++)	-	(++)	AF
Labyrinth P-TGC	(++)	(+)	PAS	(++)	-	(+)	AF
Labyrinth	(+++)	Vessel walls, in the chorionic plate+	PAS	(++)	(+)	(++)	AF
Yolk Sac	(+++) Basement membrane and epithelial surface	Basement embrane++, Epithelial surface +	PAS /AB	(+++) Basement membrane epithelial surface	(++)	(++)	(+++) Basement membrane epithelial surface

None (-), Weak (+), Medium (++), Strong (+++).

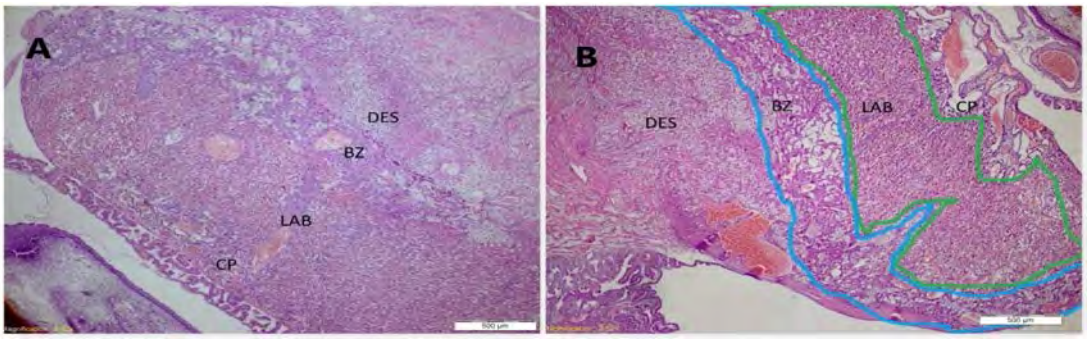

Figure 10. Seventeenth day of pregnancy. **A**-**B.** CP: Chorionic Plate, LAB: Labyrinth, BZ: Connection Zone, DES: Decidua basalis. Hematoxylin-Eosin staining method. Bar 500 µm.

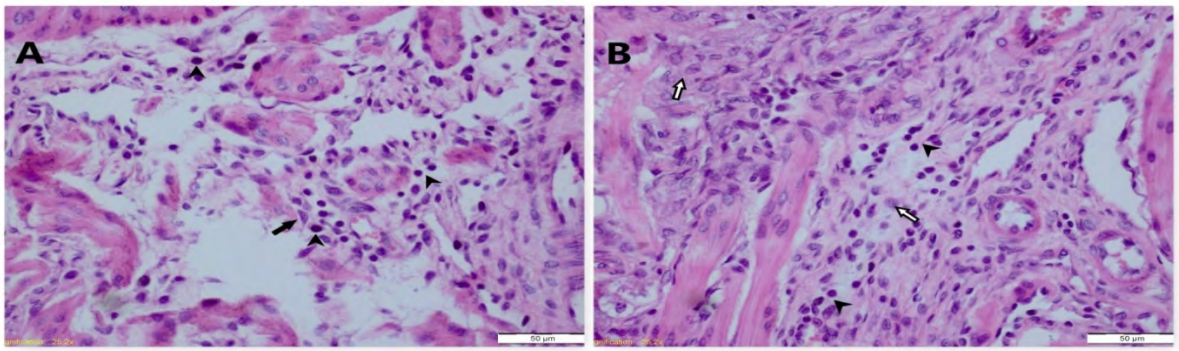

Figure 11. Seventeenth day of pregnancy**. A**-**B**. Lymphocyte infiltration (arrowheads), decidua cells (black and white arrows). Hematoxylin-Eosin staining method. Bar 50 µm.

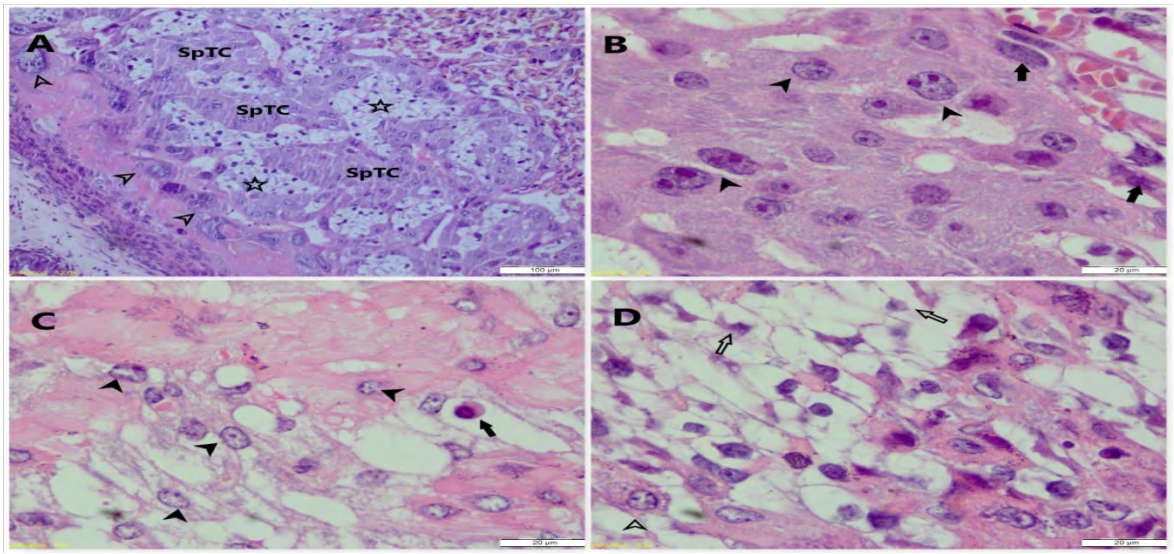

Figure 12. Seventeenth day of pregnancy. Junction zone of the placenta**. A**. SpTC: pongiotrophoblast cells, GlyC, glycogen cells (*), TGC (arrowheads). Bar 100 µm. **B**. SpTC (arrowheads), S-TGC (arrows). Bar 20 µm. C. Decidua cells (arrowheads), plasma cells (arrow). Bar 20 µm. D. GlyC (white arrow), decidua cells (white arrowhead). Hematoxylin-Eosin staining method. Bar 20 µm.

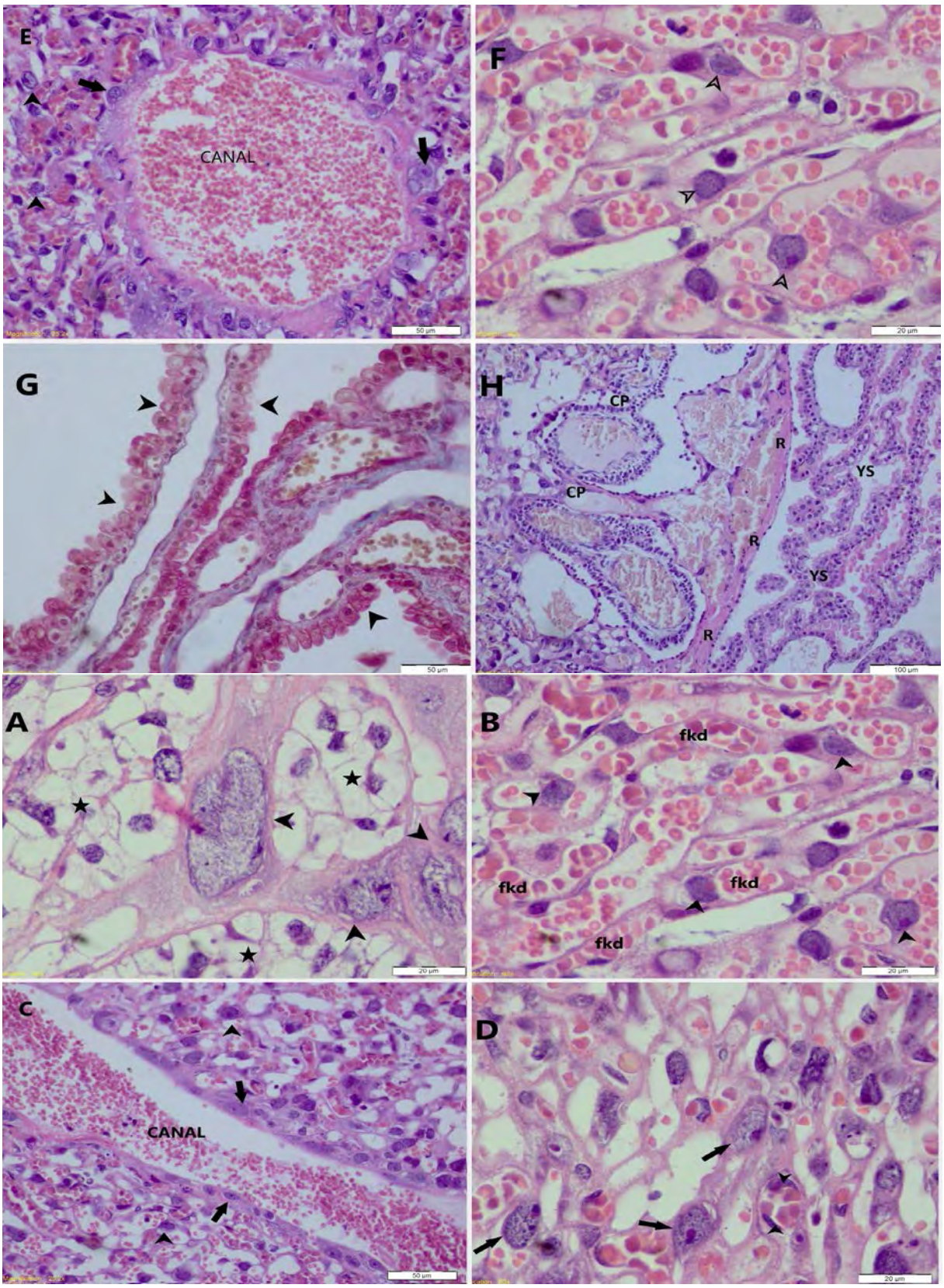

Figure 13. Seventeenth day of pregnancy. **A**. P-TGCs (arrowheads) located in the region limiting the fetal part, and GlyC communities (stars) located around it. Hematoxylin-Eosin staining method. Bar 20 µm. **B**. Labyrinth. Fetal blood vessels (fkd) and S-TGCs (arrowheads) Hematoxylin-Eosin staining method. Bar 20 µm. **C**. Channel structure in the labyrinth. C-TGCs (arrows), and S-TGCs (arrowheads) bordering the channel. Bar 50 µm. **D**. Labyrinth S-TGCs (arrows), fetal endothelial cells (arrowheads). Hematoxylin-Eosin staining method. Bar 20 µm. E. C-TGCs (arrows) on the labyrinth part canal wall. Bar 50 µm. **F**. The labyrinth part. S-TGCs (arrows). Bar 20 µm. G. Amniotic cavity, vitellus epithelial cells (arrowhead). Triple staining method.Bar 50 µm **H.** Chorionic plate region (CP) located at the end of the labyrinth section, Reichert's Membrane (R) limiting the chorionic plate, yolk sac (YS) in the amniotic cavity. Hematoxylin-Eosin staining method. Bar 100 µm.

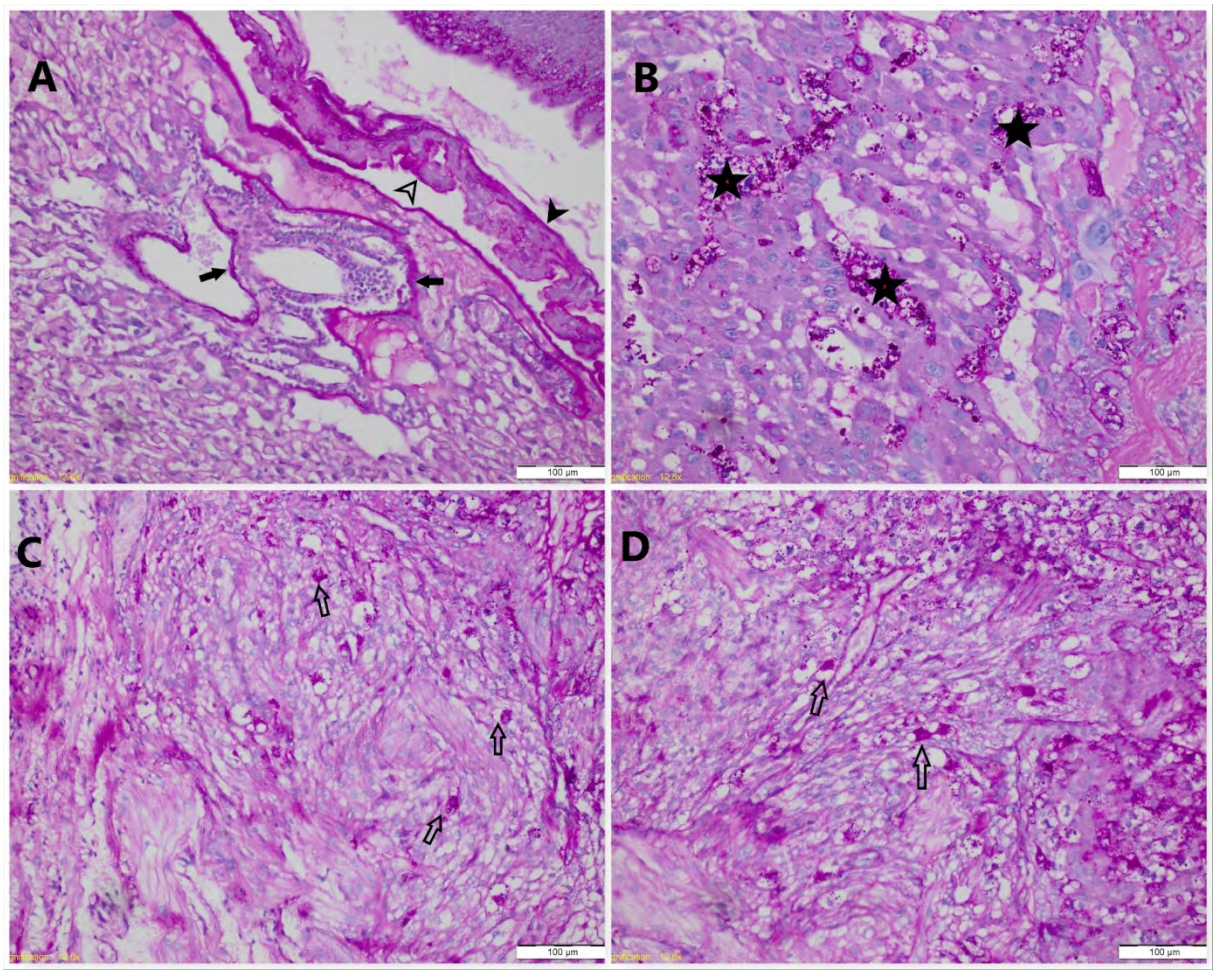

Figure 14. Seventeenth day of pregnancy. A) Strong PAS reaction on the yolk sac basement membrane (black arrowhead) and epithelial surface (white arrowhead). B) Glycogen cell populations showing a very strong granular PAS-positive reaction in the junction zone (*). C-D) PAS+ cells (open arrows) thought to be uNk cells in the MLAp-compatible region. PAS staining method. Bar 100 µm.

Discussion

Edwards et al. (2014) reported in their study that the mouse uterus looked normal microscopically in the preimplantation period. They stated that the endometrium consists of the lumen epithelium of the uterus, gland epithelium, and stroma, circular and longitudinal myometrium surrounds the endometrium, and the outermost is the perimetrium.

In the presented study, it was observed that the histological structure of the uterus was generally preserved on the fourth day. It was determined that only some parts of the endometrium showed structural deterioration and some stromal cells began to differentiate structurally.

Studies report different findings regarding the day of implantation in mice. Enders and Blankenship (1999) stated that implantation occurred on the 4.5th day. Arvola (2001) states that it occurs on the 3.5th and 4th days, and in some studies, it occurs on the 4.5th and 5th days (Deb and Paria, 2006; Rossant and Cross, 2001; Watson and Cross, 2005). Deb and Paria (2006) state that the decidualization process is a response that occurs as a result of implantation, and that implantation is the initiator of the proliferation and differentiation of uterine stromal cells. In another study, it was reported that the implantation area could be observed as early as the fifth day of pregnancy (Flores et al., 2014). In the presented study, no macroscopic or microscopic signs of implantation were found in the uterine samples taken on the fourth day of pregnancy, and no decidualization area was observed.

Sur et al. (2015) reported that decidualization begins in the mesometrial part where the implantation occurs as the implantation takes shape. It has been determined that, as decidualization progresses, parts of the mature structure of the placenta begin to form in the second and third weeks of pregnancy. The presented study observed that the placenta's main parts began to take shape from the tenth day of pregnancy, and the mature placenta structure was formed on the seventeenth day of pregnancy.

It is reported that fetal blood vessels begin to form towards the tenth day of pregnancy. These blood vessels widely invade the trophoblasts surrounding the maternal blood spaces, which can be observed on the 12th day of pregnancy (Enders and Blankenship, 1999). In this study, it was observed that on the tenth day of pregnancy, the labyrinth part began to form, maternal and fetal blood cells

could be clearly distinguished, and the maternal and fetal structures in the labyrinth were irregularly located and in the process of formation.

Flores et al. (2014) observed that on the 9th day of pregnancy, the antimesometrium, called the decidua capsularis, directly opposite the mesometrium, was considerably thinner than the mesometrium, but contained much more dense and dense cells and was much less vascularized. They also reported that nucleated fetal erythrocytes were clearly observed during this period. In the same study, when the 10th and 13th days of pregnancy were examined, it was stated that the placenta gained a mature placenta-like appearance, including the labyrinth zone, TGC zone, and spongiotrophoblast zone, and the decidua capsularis and the TGCs in between began to regress. In our study, histological findings similar to those of Flores et al. (2014) were obtained. On the tenth day of pregnancy, the antimesometrial decidua (decidua capsules) was considerably thinner and much less vascularized than the mesometrial decidua (decidua basalis). Its cellular density was much higher. Likewise, on the tenth day, fetal erythrocytes were nucleated and clearly distinguishable from maternal erythrocytes.

Edwards et al. (2014) stated that a large amniotic cavity, a prominent maternal decidua, and lumen residue were observed in the mouse uterus at day 9.5 of pregnancy. It has been stated that the MLAp part expands, is positioned between the myometrium, and divides the myometrium. It is reported that these changes occur in the mesometrial part, and the antimesometrial part is thinner. The same study stated that the mature placenta is generally formed on the 10.5th day of pregnancy, and the trophoblastic giant cell, spongiotrophoblast, and labyrinth layer can be clearly distinguished. It has been stated that TGCs terminate the labyrinth part, and nucleated fetal erythrocytes and nucleated maternal erythrocytes can be easily distinguished in the labyrinth part. It has been stated that the spongiotrophoblast layer is easily distinguished from the labyrinth, the amino space has widened considerably, and the lateral and antimesometrial decidua have begun to regress (Edwards et al., 2014). In this study, results supporting histological images were observed on the tenth day of pregnancy. It was observed that the labyrinth had begun to be established and was distinguished from the other layers, the antimesometrial layer was considerably thinner compared to the mesometrial part, the MLAp part was considerably enlarged, and nucleated fetal erythrocytes could be clearly seen in the labyrinth.

Harem and Alabay (2018) detected giant cells characterized by large nuclei and large cytoplasm, and glycogen cells clustered in the labyrinth, in the rat placenta on the fifteenth day of pregnancy. On the twentieth day, they reported that they observed giant cells, vacuolated cells in the decidual region, small basophilic cells with dark nuclei around the maternal and fetal vessels, and multinucleated trophoblast cells. The presented study found glycogen cells in the junction zone and occasionally in the decidua basalis on the seventeenth day. Giant cells were also observed in the labyrinth and the part bordering the labyrinth.

Edward et al. (2014) reported that in the mouse placenta, on the 15.5th day of pregnancy, the placenta was concentrated entirely in the antimesometrial part, and the decidua basalis and MLAp parts, which were evident in the previous days, started to regress. It is stated that the uterine cavity is completely re-epithelialized and becomes a residue. In the same study, it was reported that the placenta on the 19.5th day of pregnancy had all the histological features of a fully mature placenta, but some membranes began to appear dilated due to the last days of pregnancy. In this study, all histological layers of the mature mouse placenta could be observed on the seventeenth day of pregnancy. Consistent with the findings of the researchers, it was observed that the MLAp and decidua basalis part regressed and the placenta was located only in the antimesometrial part. On the seventeenth day, lamina epithelialis and uterine cavity were not observed.

Histochemical Evaluations: In a study conducted in rats (Mutluay, 2019), it was reported that basement membrane integrity continued and the basement membrane was PAS positive in samples taken on the fifth day of pregnancy. In the presented study, PAS positivity was observed in the basement membrane on the fourth day of pregnancy. Harem and Alabay (2018) applied PAS, AB PH 2.5, and PAS/AB staining methods to placenta samples from the sixth, tenth, fifteenth, and twentieth days of pregnancy in rats. In PAS histochemical staining, it was stated that positivity was observed in TGCs and other trophoblasts on the 10th day, and intense PAS positivity was observed in Gly-C and TGCs on the 15th and 20th days. In AB pH 2.5 staining, it was stated that on the tenth day, pale AB staining was again observed in the trophoblasts and the capsule surrounding the TGC. In PAS/AB pH 2.5 staining, it was reported that a faint AB positivity was observed in TGCs on the tenth day, PAS positivity was observed in TGCs on the fifteenth and twentieth days, and PAS positivity was also observed in trophoblasts on the twentieth day. In this study, intense PAS positivity was observed in Gly-C and TGCs, which is consistent with the study conducted on the tenth day of pregnancy. On the seventeenth day, intense PAS staining was observed in Gly-Cs. In AB pH 2.5 staining, a moderate reaction was observed in Gly-C and labyrinth, with a weak TGC on the tenth day, while an AB reaction was observed in Glyc-Cs on the seventeenth day. In a study investigating the effect of quercetin on placenta morphology in streptozotocin-stimulated diabetic rats (Erşahin et al., 2016), it was reported that as a result of PAS staining applied on the 21st day of pregnancy in control group rats, a high amount of PAS reaction was observed in the maternal part of the placenta in the control group and a low amount of PAS reaction was observed in the labyrinth zone. In this study, PAS reaction of varying intensity was observed in both the labyrinth and the maternal part in placenta samples from the seventeenth day of pregnancy.

It has been reported that PAS-positive areas are observed in the metrial region and decidua basalis in the later stages of pregnancy in mice (Sur et al., 2015). In our study, PAS-positive cells were found in the decidua basalis and mesometrial part on the seventeenth day of pregnancy.

It has been stated that glycogen cells give a positive reaction to PAS between days 6.5 and 12.5 of pregnancy (Tesser et al., 2010). In the presented study, Gly-C gave a positive reaction to PAS on the tenth and seventeenth days of pregnancy. Sur et al. (2015) reported in their study that Uterus Natural Killer Cells (uNK) cells showing intense PAS positivity were encountered in the decidua basalis in the middle and last days of pregnancy. It is stated that the number of these cells, which play a role in the restructuring of the endometrium and vessels during decidualization, is very high in the middle of pregnancy, and a significant decrease is observed towards the end of pregnancy (Sur et al., 2015). In the presented study, cells thought to be PAS-positive uNK were detected in the MLAp section on all gestational days studied. These cells were most dense on the tenth day of pregnancy.

It was reported that glycogen cells showed AB positivity after PAS-AB histochemical application applied after the de-cellularization process performed on the mouse placenta on the eighteenth and a half day of pregnancy (Barreto et al., 2019). In this study, the PAS positivity of glycogen cells was observed on the seventeenth day of pregnancy. Different AB pH 2.5 positivity levels were observed in glycogen cells in the junction zone.

As a result, this study determined the histological and histochemical characteristics of the mouse placenta during the fourth, tenth, and seventeenth days of pregnancy. This study will shed light on future studies and provide resources.

Conflict of Interest

The authors stated that they did not have any real, potential, or perceived conflict of interest.

Ethical Approval

This study was performed with the permission of the Experimental Animals Local Ethics Committee in Aydın Adnan Menderes University with (ADU HADYEK) 64583101/2016/017 approval number.

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Similarity Rate

We declare that the similarity rate of the article is 10 % as stated in the report uploaded to the system.

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References

Adams AD, Guedj F, Bianchi DW, 2020: Placental development and function in trisomy 21 and mouse models of Down syndrome: Clues for studying mechanisms underlying atypical development. *Placenta*, 89(1), 58-66.

Arvola M, 2001: Immunological aspects of maternal-fetal interactions in mice. Doctoral thesis, comprehensive summary. Acta Universitatis Upsaliensis, 13-33.

Barreto RSN, Romagnolli P, Fratini F, Mess AM, Miglino MA, 2019: Mouse placental scaffolds: a three-dimensional environment model for recellularization. *J Tissue Engin*, 10, 1-11.

Chavatte-Palmer P, Tarrade A, 2016: Placentation in different mammalian species. In *Ann D'endocrin*, 77(2), 67-74.

Culling CFA, Allison RT, Bar WT, 1985: Cellular Pathology Techniques. Butterworth & Co. (Publishers) Ltd. 113,217,232,233,238.

Deb KF, Paria BC, 2006: Methodologies to study implantation in mice. In: Soares MJ, Hunt MJ (Eds) Placenta and Trophoblast. New Jersey Springer, 470p, 9-34.

Edwards AK, Janzen-Pang J, Peng A, Tayade C, Carniato A, Yamada AT, Patricia DA, Tse D, 2014: Microscopic Anatomy of the Pregnant Mouse Uterus Throughout Gestation. In: Croy BA, Yamada AT, DeMayo FJ, Adamson SL(eds.), The Guide to Investigation of Mouse Pregnancy, Academic Press, Boston, 43-69.

Enders AC, Blankenship TN, 1999: Comparative placental structure. *Advanced Drug Delivery Reviews*, 38(1), 3-15.

Erşahin A, Hocaoğlı M, Demirer S, Cengiz F, 2016: Kuersetin, streptozotosin uyarımlı diyabetik sıçanlarda metabolik devreleri ve plasenta morfolojisini iyileştirmektedir. *Perin Derg*, 24(3), 147-55.

Flores LE, Hildebrandt TB, Kühl AA, Drews B, 2014: Early detection and staging of spontaneous embryo resorption by ultrasound biomicroscopy in murine pregnancy. *Repr Biol Endocrin*, 12(1), 38.

Furukawa S, Hayashi S, Usuda K, Abe M, Hagio S, Ogawa I, 2011: Toxicological pathology in the rat placenta. *J Toxicol Pathol*, 24(2), 95-111.

Furukawa S, Kuroda Y, Sugiyama A, 2014: A Comparison of the histological structure of the placenta in experimental animals. *J Toxicol Pathol*, 27(1), 11-18.

Hafez S, 2017: Comparative Placental Anatomy: Divergent Structures Serving a Common Purpose. *Progress in Molecul Biol Trans Sci*, 145, 1-28.

Harem İŞ, Alabay B, 2018: Ratlarda gebeliğin değişik dönemlerinde trofoblastların özellikleri üzerinde histokimyasal ve immunohistokimyasal çalışmalar. *Harran Üniv Vet Fak Derg*, (Özel Sayı), 1-11.

Johnson MH, Everitt BJ, 2018: Coitus and fertilization. In: Essential reproduction. John Wiley & Sons, United Kingdom, 145-160.

Koç S, Kum S, 2022: Investigation of IgG, vimentin, CD45 distribution, and density in mouse placenta at different periods of pregnancy. *Turk J Vet Anim Sci*, 46,505-516.

McGeady TA, Quinn PJ, FitzPatrick ES, Ryan MT, Kilroy D, Lonergan P, 2017: Fertilization In: Veterinary Embryology, University of Minnesota, 400, 26-100.

Mutluay D, 2019: Preimplantasyon sürecinde sıçan uterus dokusunda meydana gelen değişikliklerin ışık mikroskobik düzeyde incelenmesi. *MAE Sağlık Bilimleri Enst. Derg*, 3(2), 43-53.

Özer A, 2007: Veteriner Embriyoloji.Nobel Yayın Dağıtım, Ankara, 27-41.

Rai A, Cross JC, 2014: Development of the hemochorial maternal vascular spaces in the placenta through endothelial and vasculogenic mimicry. *Develop Biol*, 387(2), 131-41.

Rossant J, Cross JC, 2001: Placental development: lessons from mouse mutants. *Nature Rev Gen*, 2(7), 538.

Sinowatz F, 2009: Fertilization. In: Hyttel P, Sinowatz F, Vejlsted M, Betteridge K (eds), Essentials of domestic animal embryology. Elsevier Health Sciences, Canada, 56-68.

Sur E, Öznurlu Y, Özaydin Y, Çelik İ, Aydın İ, Kadyralieva N, 2015: Gebe farelerde desidua bazalis dokusundaki PAS-pozitif uterus doğal katil hücrelerinin dağılımı. *Kafkas Univ Vet Fak Derg*, 21(3), 405-411.

Tesser RB, Scherholz PLA, Nascimento L, Katz SG, 2010: Trophoblast glycogen cells differentiate early in the mouse ectoplacental cone: putative role during placentation. *Histochem Cell Biol*, 134, 83-92.

Tewari V, Tewari A, Bhardwaj N, 2011: Histological and histochemical changes in placenta of diabetic pregnant females and its comparison with normal placenta. *Asian Pacific J Trop Dis*, 1(1), 1-4.

Watson ED, Cross JC, 2005: Development of structures and transport functions in the mouse placenta. *Physiol*, 20, 180-93.

Wu D, Xu J, Lei J, McLane M, Zijl PC, Burd I, 2018: Dynamic glucose enhanced MRI of the placenta in a mouse model of intrauterine inflammation. *Placenta*, 69, 86-91.

Yu Q, Qiu Y, Wang X, Tang J, Liu Y, Mei L, Li M, Yang M, Tang L, Gao H, Zhang Z, Xu W, He Q, 2018: Efficient siRNA transfer to knock down a placenta-specific lncRNA using RGD-modified nano-liposome: A new preeclampsia-like mouse model. *Inter J Pharm*, 546(1-2), 115-24.

Zhu H, Xu X, Shi X, Feng Y, Xiong Y, Nan Y, Zhang C, Gao L, Chen Y, Xu D, Wang H, 2019: Activation of autophagy inhibits cadmium-triggered apoptosis in human placental trophoblasts and mouse placenta. *Environ Poll*, 254(PtA), 112991.

HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Histological Typing and Morphological Characterization of Canine Seminomas

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Abstract: The aim of this study was to evaluate seminomas based on cell morphology and histological structures and classify them into subtypes. Thirty canine seminomas were stained with hematoxylin-eosin (HE) and Periodic Acid Schiff (PAS). PAS-positive cases were classified as Classical seminoma (CS) (n:5), while PAS-negative cases were categorized as Spermatocytic seminoma (SS) (n:23). Cases exhibiting both positive and negative staining were evaluated as Spermatocytic/Classical seminoma (n:2). Carcinoma in situ were observed in the cases with Classical seminoma (n:3). The cell morphology and mitotic indices of each case were given in detail. Canine seminomas were classified into three subtypes: intratubular (n:12), diffuse (n:1), and intratubular/diffuse (n:10). As a result, it was thought that SS and CS types could be observed together, and it was observed that the intratubular/diffuse subtype is a third type characterized by its transformation from intratubular to diffuse form.

Keywords: *Canine seminoma, Classical seminoma, Histopathology, Spermatocytic seminoma.*

Köpek seminomlarının histolojik tiplendirilmesi ve morfolojik karakterizasyonu

Özet: Çalışmanın amacını, seminomları hücre morfolojisi ve histolojik yapılarına göre değerlendirerek alt tiplerine ayırmak oluşturdu. Otuz adet seminom hematoksilen-eozin (HE) ve Periyodik Asit Schiff (PAS) ile boyandı. PAS pozitif vakalar Klasik seminom (CS) (n:5), PAS negatif vakalar ise Spermatositik seminom (SS) (n:23) olarak sınıflandırıldı. Hem pozitif hem de negatif boyanma gösteren vakalar Spermatositik/Klasik seminom (n:2) olarak değerlendirildi. Klasik seminom vakalarında Carcinoma in situ gözlendi (n:3). Her bir vakaya ait hücre morfolojisi ve mitotik indeksleri ayrıntılı olarak verildi. Köpek seminomları üç alt tipe ayrıldı: intratübüler (n:12), diffüz (n:1) ve intratübüler/diffüz (n:10). Sonuç olarak, SS ve CS tiplerinin bir arada görülebileceği düşünüldü ve intratübülerden diffüz forma dönüşümüyle karakterize edilen üçüncü bir tipin intratübüler/diffüz alt türü olduğu gözlendi.

Anahtar Kelimeler: *Histopatoloji, Klasik seminom, Köpek seminomları, Spermatositik seminoma.*

Introduction

Seminomas in dogs are the most frequently encountered tumors in testicular germ cell tumors, originating from germ cells within the seminiferous tubules. While they are more commonly observed in older dogs, certain breeds such as Boxers, German Shepherds, Maltese, and Norwegian Elkhounds are reported as predisposed. Cryptorchidism is also highlighted as a significant predisposing factor in the development of seminomas (Agnew and MacLachlan, 2016; Hernández-Jardón et al., 2022).

In humans, seminomas are classified into two subtypes according to the WHO: Classical Seminoma (CS) and Spermatocytic Seminoma (SS) (Mostofy and Sesterhenn,1998). Classical Seminoma, also called Typical Seminoma, is observed in young men and clinically described as aggressive and malignant. On the other hand, the second subtype, Spermatocytic Seminoma, is less malignant and more commonly observed in older men (Agnew and MacLachlan, 2016; Grieco et al., 2007; Mostofy and Sesterhenn,1998).

The difference in biological behaviour is due to the differences in the cells from which they originate. CS originates from gonocytes, while SS originates from mature spermatocytes. Gonocytes are progenitor cells that play a role in the development of prespermatogonia and spermatogonia, and they stain positively with periodic acid-Schiff (PAS) due to the presence of glycogen. For distinguishing between the two types, PAS staining and immunohistochemical expression of placental alkaline phosphatase (PLAP) can be used (Agnew and MacLachlan, 2016; Grieco et al., 2007).

The initial stage of CS, considered as a preneoplastic lesion or referred to as "carcinoma in situ (CIS)," consists of embryonic morphology composed of PAS and PLAP-positive germ cells. As a result, CIS structures are not observed in SS. In human medicine, although the CIS structure is typically observed in CS, it is believed to be the precursor of most germ tumors (Agnew and MacLachlan, 2016; Grieco et al., 2007; Skakkebaek et al., 1987).

In domestic animals, seminomas are histomorphologically categorized into two forms: intratubular and diffuse. If neoplastic germ cells are located within the seminiferous tubules, it is classified as intratubular; if they are spread throughout the entire field independent of tubules, they are considered diffuse. Canine seminomas are morphologically more compatible with SS. SS exhibit neoplastic germ cells with varying sizes, indistinct borders, amphophilic cytoplasm, vesicular nuclei, and prominent nucleoli. Giant cells and mitotic figures are frequently observed in the SS type. Due to the filamentous chromatin appearance, typical "spiremes" mitosis is commonly encountered in the SS type. Despite being intratubular, CIS should be distinguished with the help of PAS-positive staining and cellular morphology from Spermatocytic Seminoma (SS). On the other hand, Classical Seminoma (CS) cells are of a single type and are monomorphic. They have abundant cytoplasm with distinct borders and show PAS and PLAP-positive staining (Agnew and MacLachlan, 2016; Grieco et al., 2007; Kennedy et al., 1998).

The comprehensive aim of the study was to evaluate canine seminomas. In the study, the investigation and comparison of 30 cases of seminoma were conducted, considering the existing classifications and subtyping methods.

Materials and Methods

This study is not subject to HADYEK permission in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees".

A total of 30 testicular tissues diagnosed as seminoma at Ankara University Veterinary Faculty Pathology Department were included in the study. Among these, 27 were operation materials and 3 were obtained post-mortem (necropsy material). Paraffin-embedded blocks of the testicles were cut at a thickness of 4-5 µm using a microtome, and after routine processes, they were stained with hematoxylin-eosin (HE) and Periodic Acid Schiff (PAS). Based on PAS staining, those that stained positive were classified as “Classical Seminoma (CS)”, while negative staining indicated “Spermatocytic Seminoma (SS)”. In cases where both negative and positive staining were observed in certain proportions, a modified subtyping was used as “Classical/Spermatocytic Seminoma (CS/SS)”. Cellular structures were examined in detail. For this purpose, the morphological variation in tumor cells (monomorphic/polymorphic), the clarity of their boundaries (indistinct border/abundant border), and the cytoplasmic characteristics (abundant cytoplasm/amphophilic less abundant cytoplasm) were examined. The presence of giant cells and spiremes mitosis were noted. The mitotic index was assessed by counting the mitotic figures present in 10 fields (x400). If the total number of mitotic figures in 10 random fields was less than 10, it was scored as “1”; between 10 and 50, it was scored as “2”; and if greater than 50, it was scored as “3”. As for histological patterns, they were classified according to the animal World Health Organization (WHO) as “intratubular” or “diffuse” (Kennedy et al., 1998). Tubular structures that transitioned to a diffuse form due to invasion were designated as “intratubular/diffuse”. Tumor germ cells, which morphologically resemble the classical type, that lined the tubular structures were PAS-positive and evaluated as carcinoma in situ (CIS).

Results

In the study, seminomas were most commonly observed in the following breeds, respectively: Terrier (n:10), mixed breeds (n:3), German Shepherd (n:2), Boxer (n:2), Golden Retriever (n:2), Bulldog (n:1), Pekingese (n:1), Doberman (n:1), and Akita (n:1). Breed information was not available for seven dogs. Except for one case aged three

years, seminomas were observed between the ages of 7 and 21, with an average age of 11.6 years. In our study, only two of the testicles with seminoma were cryptorchids, and one of them was observed at the necropsy. Information on the breed, age, cryptorchidism, material data, and diagnoses was given in the table (Table 1).

Table 1. Breed, age, material data of the case.

Case Number	Dog data		Material Data		Tumor Type
	Breed	Age	Cryptorchidism	Operation/ Necropsy material	
1.	Bulldog	3	Cryptorchidic testicle	Necropsy	CS/SS
2.	(N)	13	Testicle (N)	Operation	SS
3.	Mix	11	Testicles	Operation	CS
4.	Terrier	12	Testicle (N)	Operation	SS
5.	Golden retriever	7	Right Cryptorchidic testicle	Operation	SS
6.	Pekingese	13	Testicle (N)	Operation	SS
7.	Doberman	11	Testicle (N)	Operation	SS
8.	Golden retriever	9	Testicles (N)	Operation	SS
9.	Terrier	15	Testicles (N)	Operation	SS
10.	(N)	9	Testicles (N)	Operation	SS
11.	Terrier	(N)	Left testicle	Operation	SS
12.	Akita	11	Left testicle	Operation	SS
13.	German shepherd	9	Testicles (N)	Operation	SS
14.	(N)	9	Testicle (N)	Operation	CS
15.	Terrier	21	Testicle (N)	Operation	SS
16.	(N)	10	Testicle (N)	Operation	SS
17.	(N)	12	Testicle (N)	Operation	SS
18.	Jack Russell terrier	8	Testicle (N)	Operation	CS/SS
19.	(N)	8	Scrotum	Operation	SS
20.	(N)	13	Testicles	Operation	SS
21.	Terrier	13	Testicles	Operation	SS
22.	Boxer	12	Testicles	Operation	CS
23.	Yorkshire terrier	14	Testicle (N)	Operation	SS
24.	Mix	(N)	Testicle (N)	Operation	CS
25.	Mix	13	Testicle (N)	Operation	SS
26.	Terrier	9	Left testicle	Operation	SS
27.	Terrier	13	Testicle (N)	Operation	SS
28.	Terrier	13	Testicle (N)	Operation	SS
29.	Boxer	10	Right testicle	Necropsy	CS
30.	German shepherd	13	Testicle (N)	Necropsy	SS

(N): No-information; Testicle **(N):** No-information about whether they are a right or left testicle.

After PAS staining, cases with germ cells showing red-stained cytoplasm, PAS-positive, were classified as Classical seminoma (n: 5) (Figure 1), while those without staining, PAS-negative, were categorized as Spermatocytic seminoma (n: 23) (Figure 2). In two cases of seminoma, despite a morphological appearance closer to the spermatocytic classification, a certain degree of PAS-positive staining was observed. Therefore, these were indicated as Spermatocytic/Classical seminoma (n: 2) (Figure 3).

Classical seminomas (CS): The animals diagnosed with classical seminoma were in the age range of 9-12 years, with the breed of one case unknown, while two were of the Boxer breed and two were of mixed breed dogs. The tumor germ cells had a single morphological type. Germ cells with

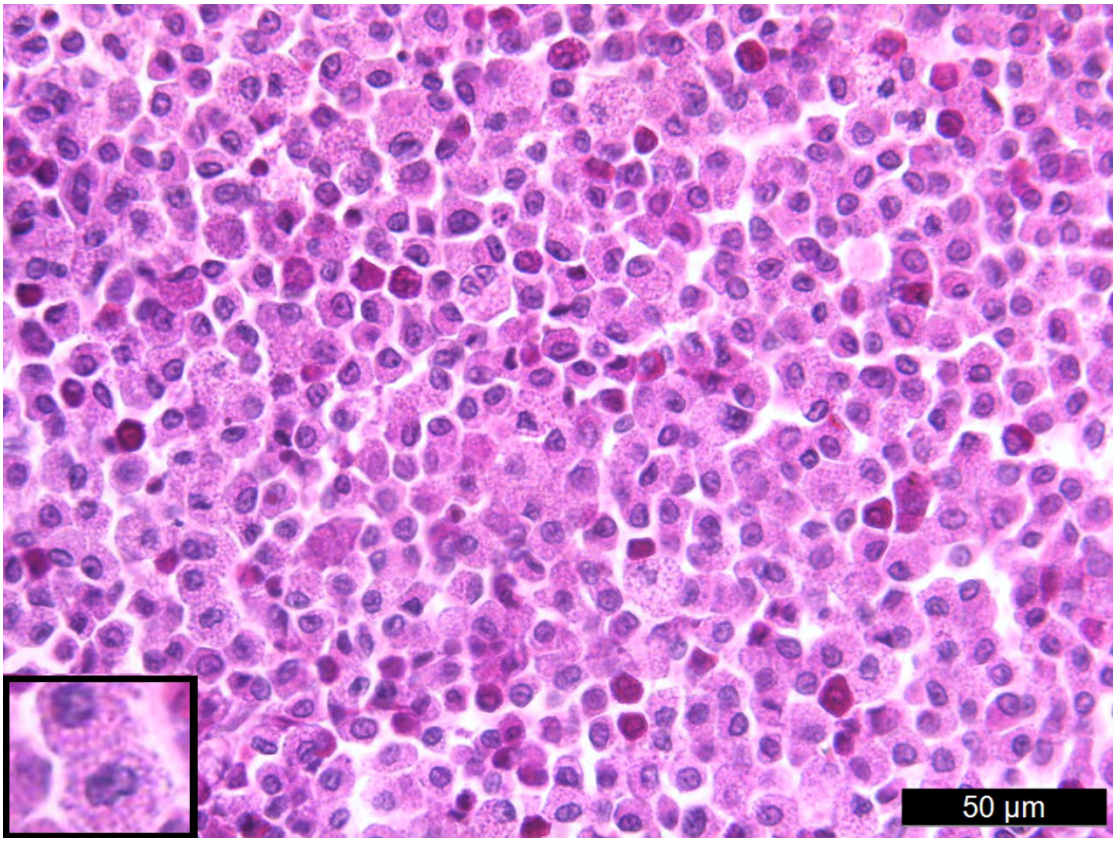

Figure 1. Classical seminoma (CS). Diffuse type seminoma composed of monomorphic neoplastic germ cells with PAS positive abundant cytoplasm (inset) and distinct border, PAS.

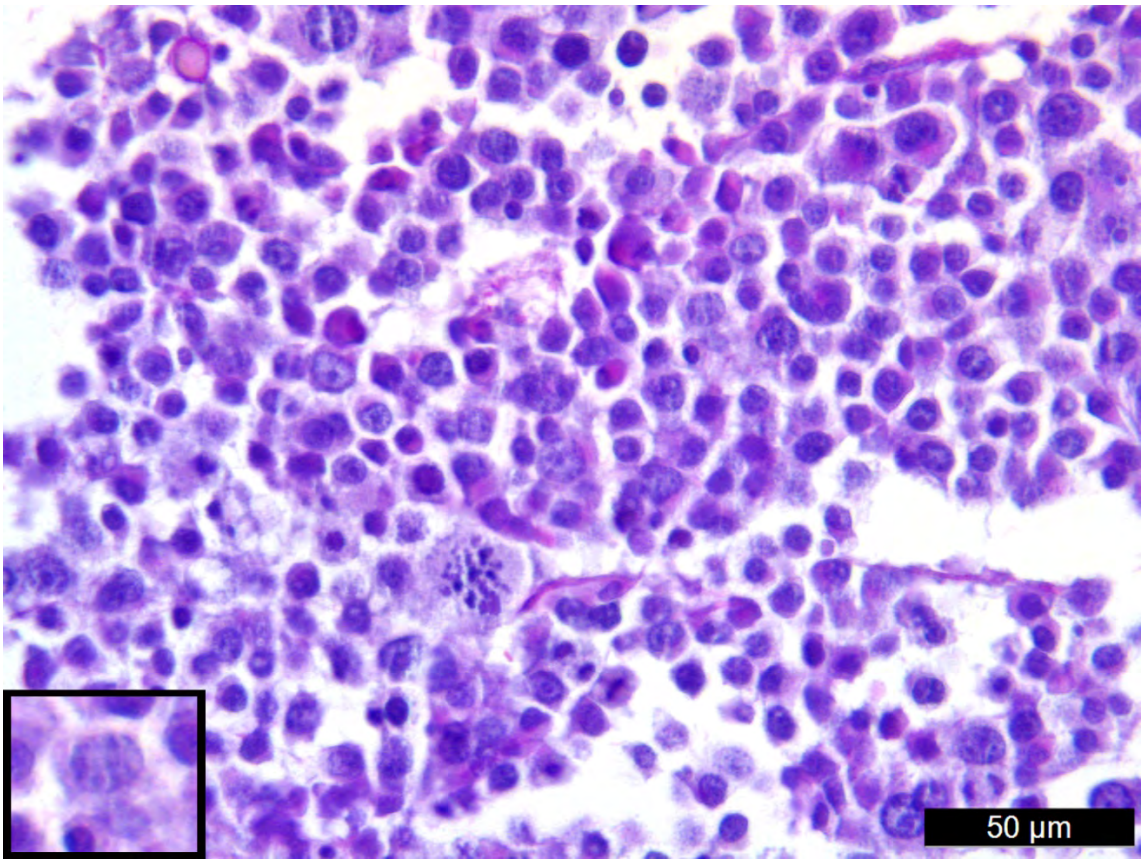

Figure 2. Spermatocytic seminoma (SS). Polymorph neoplastic cells with less abundant and PAS negative cytoplasms (inset), PAS.

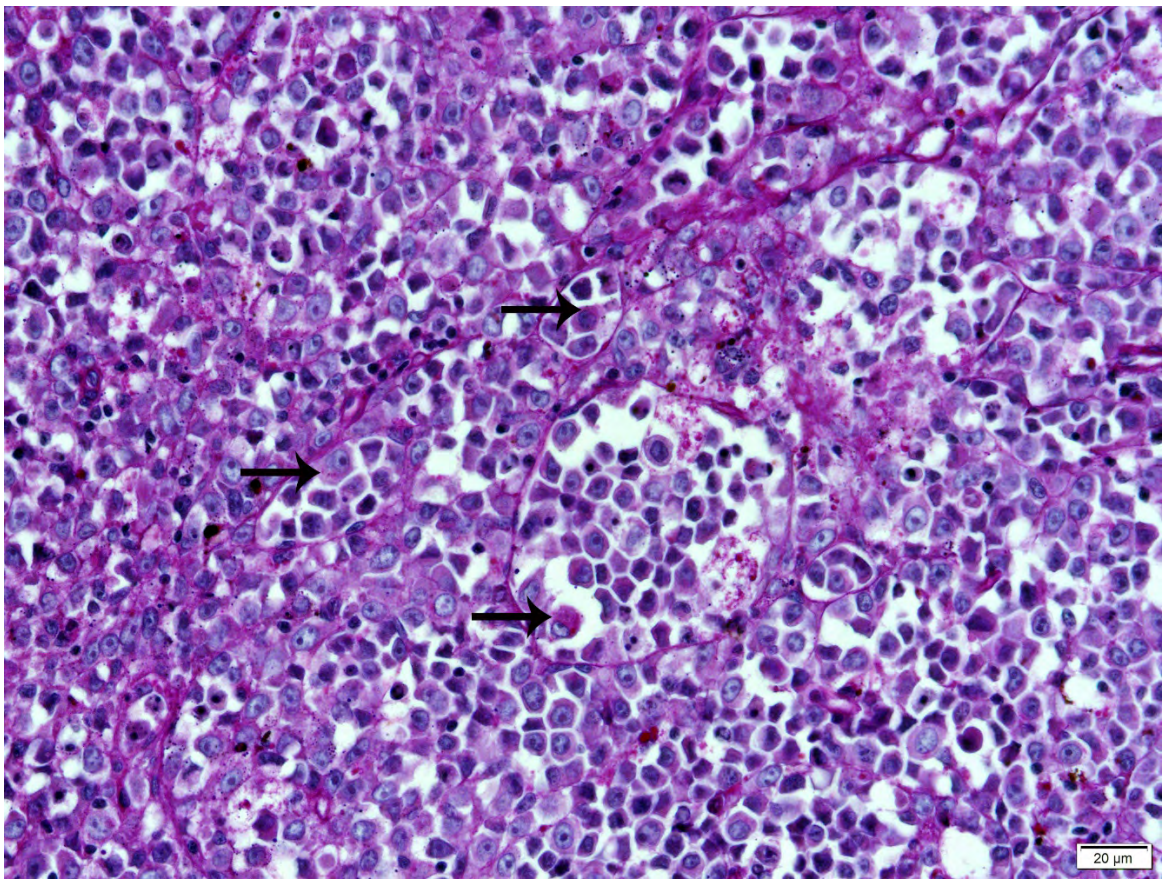

Figure 3. Spermatocytic/Classical Seminoma (SS/CS). The mixed type, consisting mainly of negative polymorphic neoplastic cells, and a few PAS-positive neoplastic cells with wide cytoplasm (arrows), PAS.

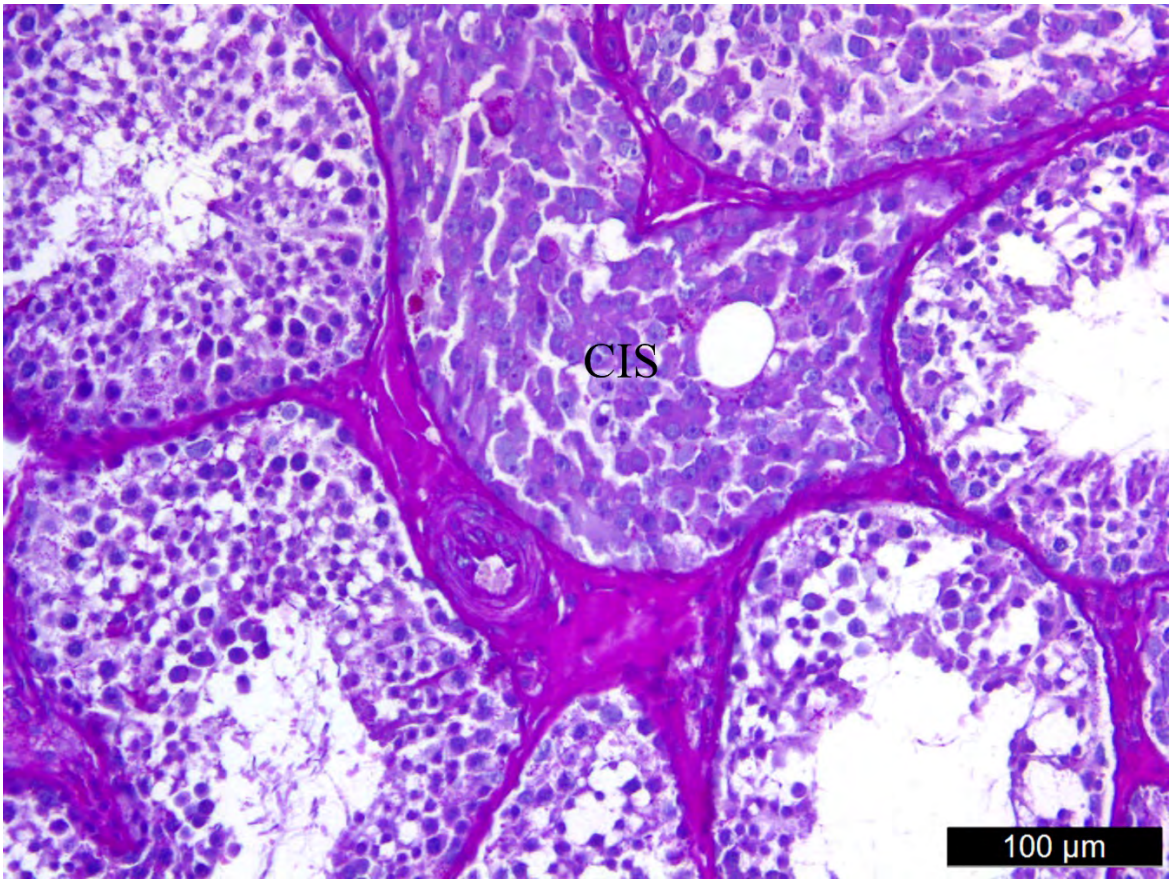

Figure 4. Testicular carcinoma in situ (CIS). Seminiferous tubule (CIS) filled by neoplastic spermatogonia, PAS.

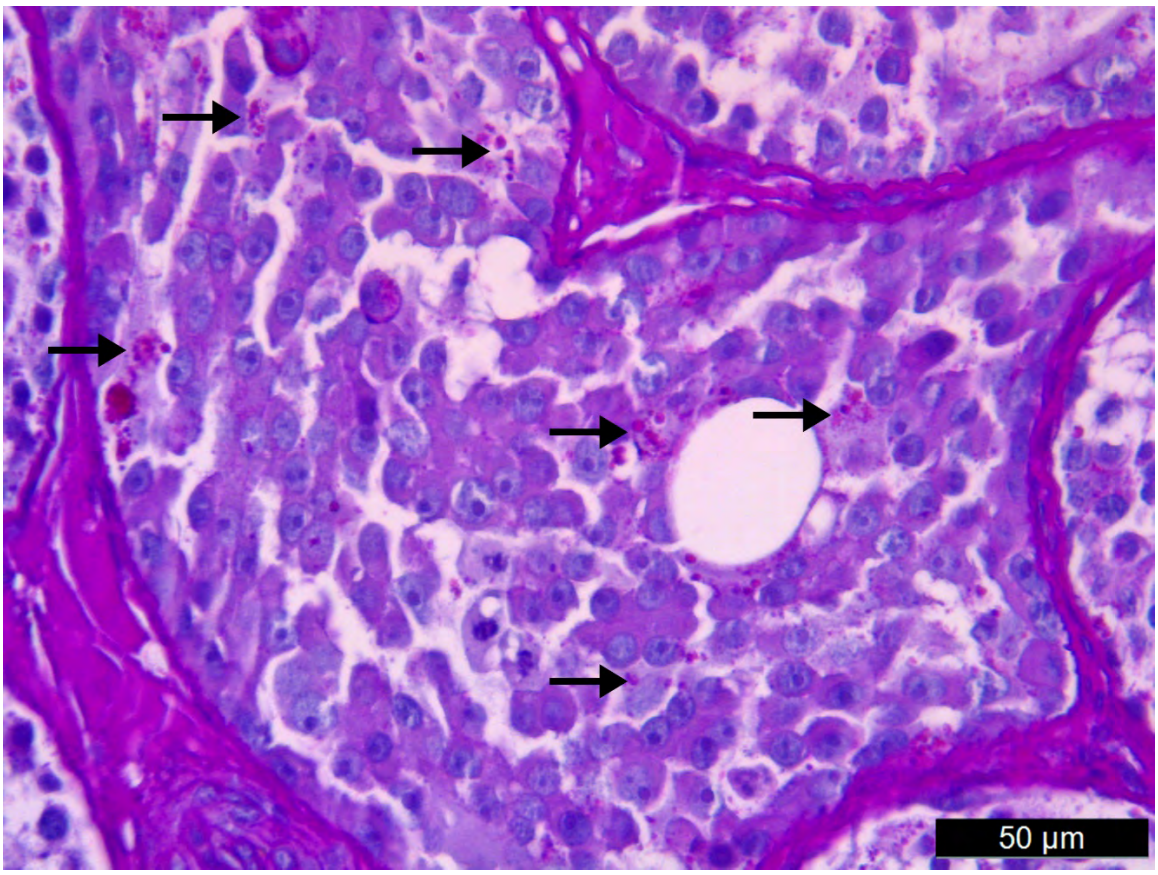

Figure 5. Testicular carcinoma in situ (CIS). Seminiferous tubule filled by neoplastic spermatogonia with PAS positive abundant cytoplasm (arrows) with well-defined boundaries, PAS.

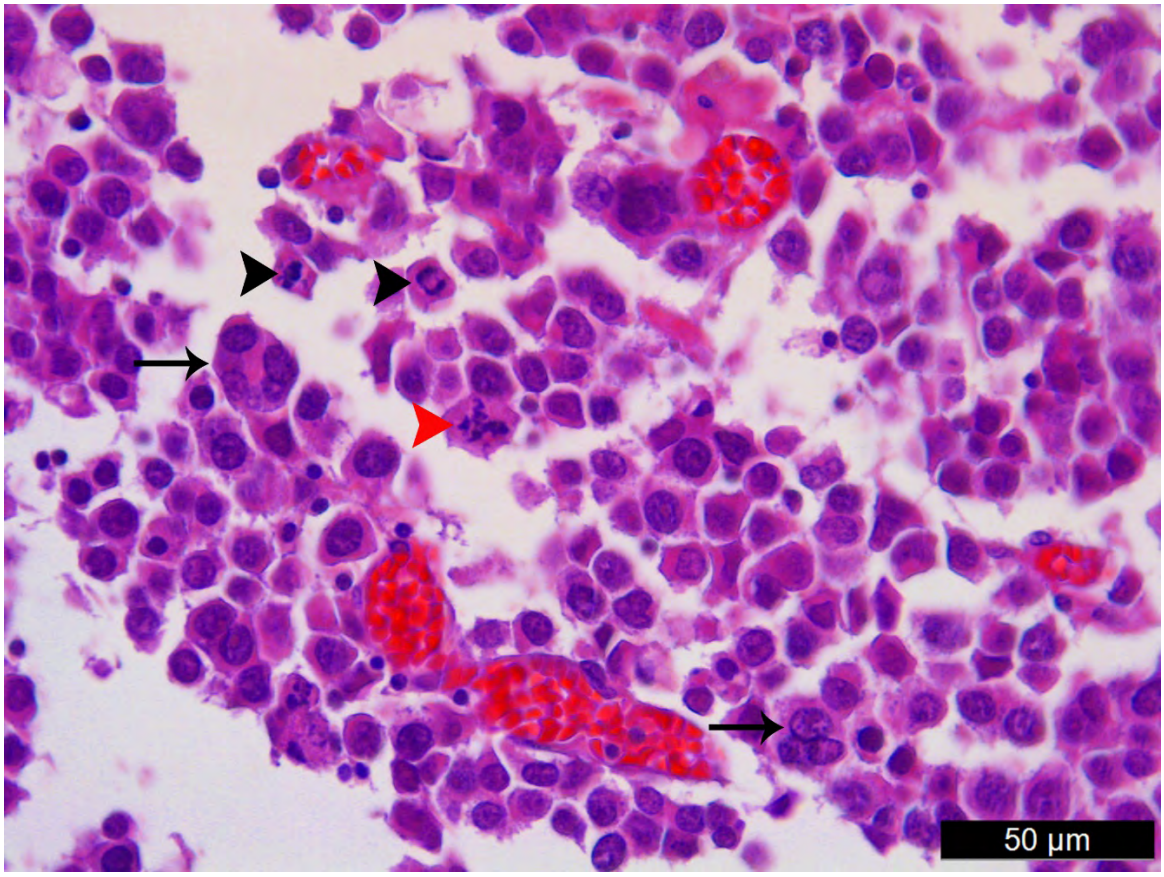

Figure 6. Spermatocytic seminoma. Giant cells (black arrows), mitosis (arrowheads) with spiremes type (red arrow), HE.

eosinophilic, abundant cytoplasm had well-defined boundaries. Giant cells were not observed in any case except for one. In all cases, the mitotic index was 1, and only one case showed a spiremes mitosis. While three cases had a diffuse pattern, an intratubular pattern was observed in two cases. Both cases with intratubular patterns were evaluated as Carcinoma in situ (CIS) (Figure 4 and 5).

Spermatocytic seminomas (SS): Animals classified as spermatocytic seminomas were in the age range of 7-21 years, with the majority observed in terrier-breed dogs (n=10). Among most spermatocytic seminoma cases, notable differences in cell size were observed among germ cells, leading to a characterization of polymorphism. Only two cases displayed a monomorphic appearance. Germ cells with amphophilic, less abundant cytoplasm had indistinct boundaries. Distinct cytoplasmic tumor cells were identified in one case. Giant cells were found in most cases (n=15). The mitotic index was mostly scored at 2 (n=12), and numerous spiremes mitotic figures (n=14) were encountered (Figure 6). Among the SS cases, the majority (n=12) displayed a typical intratubular pattern (Figure 7). In the remaining cases (n=10), although a diffuse appearance predominated, it was observed that the intratubular pattern turned into a more diffuse pattern due to tubular invasion, leading to an evaluation of the intratubular/diffuse pattern (Figure 8). One case exhibited only a diffuse pattern.

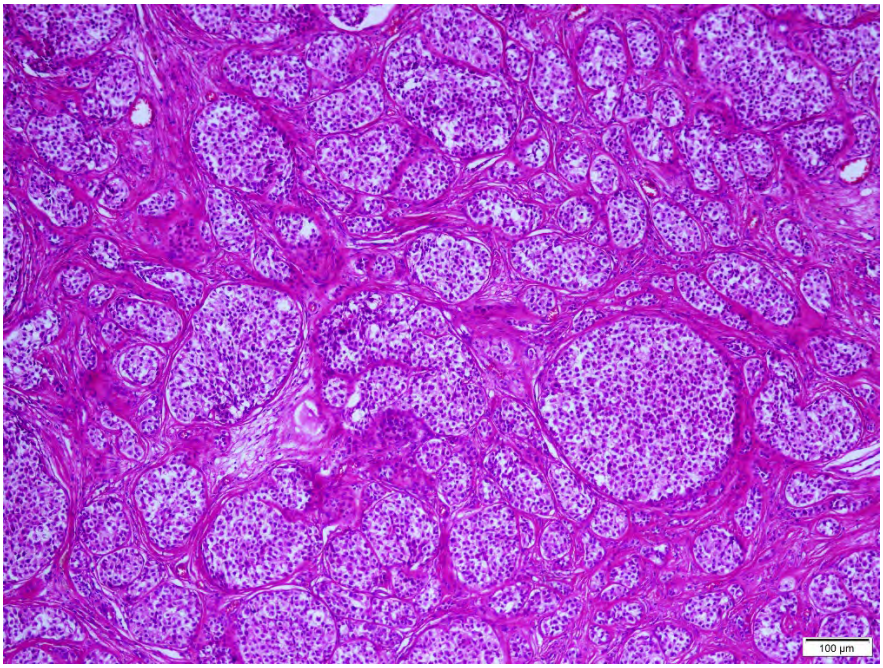

Figure 7. Spermatocytic seminoma. Intratubular type composed of neoplastic germ cells inside tubules, HE.

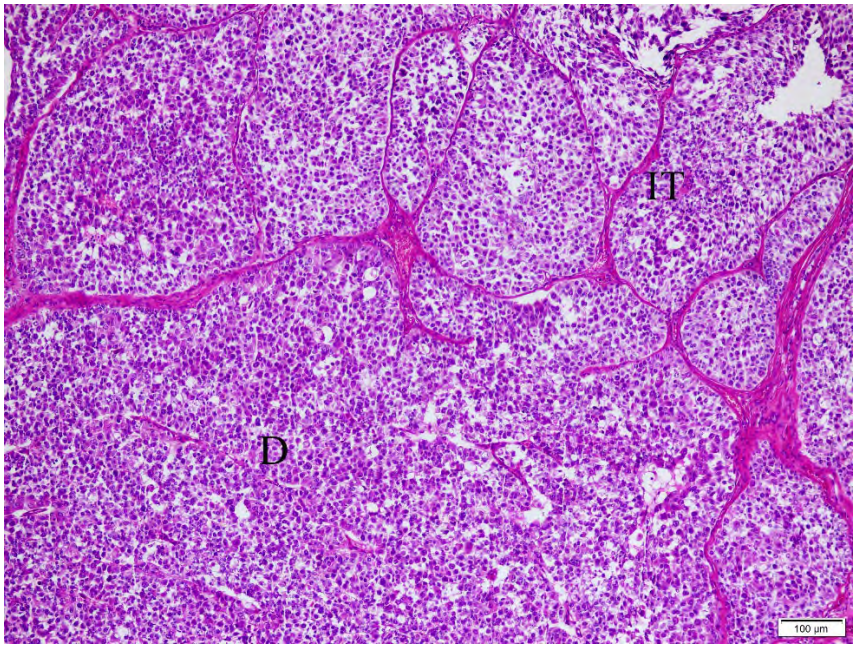

Figure 8. Spermatocytic seminoma. Intratubular/diffuse type composed of diffuse (D) and intratubular type (IT), HE." kullanılmalı.

Table 2. Tumor cell morphology, tumor structures and types of the cases.

Case Number	Tumor cell morphology									Tumor structure				Tumor Type
	Monomorphic	Polymorphic	Well defined border	Indistinct border	Abundant eosinophilic cytoplasm	Amphophilic less abundant cytoplasm	Present of giant cell	Mitotic index	Spireme mitosis	Intratubular	Diffuse	Intratubular /Diffuse	Present of CIS	
1.		+	+	+	+	+	+	3	+			+		SS/CS
2.		+		+		+		3	+		+			SS
3.	+		+		+			1				+		CS
4.		+		+		+		2	+	+				SS
5.		+		+		+	+	2	+			+		SS
6.		+		+		+	+	2	+			+		SS
7.		+		+		+	+	2	+			+		SS
8.		+		+		+	+	2		+				SS
9.		+		+		+	+	2	+	+				SS
10.	+			+		+		1		+				SS
11.		+		+		+		2	+	+				SS
12.		+		+		+		1		+				SS
13.		+			+	+	+	2	+	+				SS
14.	+		+		+			1			+		+	CS
15.		+		+		+	+	2		+				SS
16.		+		+		+		1				+		SS
17.		+		+		+	+	1				+		SS
18.		+		+		+	+	2	+		+			SS/CS
19.		+		+		+	+	2	+	+				SS
20.		+		+		+	+	1		+				SS
21.		+		+		+	+	2	+			+		SS
22.	+		+		+			1		+			+	CS
23.	+			+		+		1		+				SS
24.	+		+		+			1	+		+			CS
25.		+		+		+		1	+			+		SS
26.		+		+		+	+	3	+			+		SS
27.		+		+		+	+	1		+				SS
28.		+		+		+	+	2	+			+		SS
29.	+		+		+		+	1		+			+	CS
30.		+		+		+	+	2	+			+		SS

Spermatocytic/Classical seminomas (SS/CS): In two cases, prominent PAS-positive staining was observed, mostly PAS-negative. Notably, the cell morphology of the seminoma observed in the cryptorchid testis of a three-year-old animal exhibited features of both CS and SS classifications. The mitotic indices for both were 3, and they included giant cells and spiremes mitoses. One case exhibited a diffuse pattern, while the other displayed an intratubular/diffuse pattern.

Seminoma type and details regarding cell morphology of 30 cases of seminoma were given in the table (Table 2).

Discussion

The present study aimed to comprehensively classify and subtype 30 tumor cases using both Animal and Human World Health Organization International Histological Classifications (WHO) criteria. According to human criteria, seminomas are divided into two categories: classical seminoma (CS) and spermatocytic seminoma (SS) (Mostofy and Sesterhenn, 1998). Similar to a study conducted by Maiolino et al. (2004), these criteria were used to classify CS and SS subtypes in the present study. Additionally, we performed CS and SS subtyping using PAS staining like Grieco et al. (2007). In human medicine, CS was reported to be more common, while in veterinary medicine, SS was more frequently observed. In this present study, SS was the most prevalent seminoma subtype at 76.6%. In two cases, where negative staining was predominant but there was also a significant amount of positive staining, a third subtype was called as a mixed type (SS/CS). Particularly in one case, mixed cell morphology of both types was evident, suggesting that seminomas might originate concurrently from gonocytes and spermatocytes. This case in the present study, occurring in a young 3-year-old animal with a cryptorchid testis, raised the possibility of genetic predisposition and susceptibility to different, more aggressive tumor transformations under temperature influences. Exposure to this kind of effect such as post-natal radiation, chemicals have been reported to play an important role in the pathogenesis of CS (Grieco et al., 2007; Kristensen et al., 1996; Lamb et al., 1981). We recommend performing immunohistochemical PLAP staining in addition to PAS staining in cases where mixed cases (SS/CS) are suspected.

In human medicine, it has been reported that cases of CIS are only histopathologically observed in CS and not seen in cases of SS (Agnew and MacLachlan, 2016; Bush et al., 2011). In the present study, CIS formations were also observed in only CS cases. Similarly, studies have reported that CIS structures are the precursors of most germ tumors (Bush et al., 2011; Grieco et al., 2007; Skakkebaek et al., 1987). Even Grieco et al. (2007) mentioned that such CIS cases could be observed in other seminal tumors, suggesting a maldevelopment of testis and seminal epithelium. In this study, we thought that if there were more early diagnosis possibilities for SS/CS mixed cases, we could also encounter CIS cases in SS.

According to the animal classification, seminomas are subtyped based on their features into two distinct forms: intratubular and diffuse (Agnew and MacLachlan, 2016; Nielsen, 1998). Spermatocytic seminomas show intratubular growth pattern. Diffuse-type seminomas are more aggressive and are commonly encountered as the classic type in young men (Agnew and MacLachlan, 2016; Kennedy et al., 1998; Mostofy and Sesterhenn,1998). In our study, it was noteworthy that in 43.4% of SS cases, intratubular forms transformed to diffuse form by invasion and in fact, exhibited a predominantly diffuse structure. Parallel to the study conducted by Maiolino et al. (2004), the appearance in our study resembled the diffuse-dominant intratubular/diffuse pattern upon invasion and it is closer to the Classical form seen in humans. However, contrary to the findings of previous studies (Grieco et al., 2007; Grieco et al., 2008; Nascimento, 2020), only a small number of SS cases exhibited a solo diffuse pattern.

With a few exceptions, the cytomorphological appearances were consistent with findings from other studies and literature information (Agnew and MacLachlan, 2016; Akin et al., 2013; Bush et al., 2011; Grieco, 2007; Maoilino et al.,2004; Özsoy and Kutsal, 2007; Yumusak et al., 2014). However, unlike in human medicine, CS cases were not observed in younger animals (Agnew and MacLachlan, 2016). The polymorphic appearance, presence of giant cells, spiremes mitosis and high mitotic index were observed in SS forms, while the presence of a wide cytoplasm and monomorphic appearance with distinct borders were noted in CS cases, aligning with recent research and literature knowledge (Agnew and MacLachlan, 2016; Grieco, 2007; Maoilino, 2004). In the two cases with CS/ SS mixed subtype, it was noteworthy that the mitotic index score was consistently the highest (score:3). This aspect was striking and suggested that the mixed subtype might be even more aggressive.

The age and breed range also aligned with the findings from previous studies and literature information (Agnew and MacLachlan, 2016; Grieco, 2007; Maoilino, 2004). However, the observation of 4 cases of CS in our study, with 2 in mixed breed dogs and 2 in Boxer breed dogs, while not statistically significant, suggested the need for considering the Boxer breed in this aspect.

In conclusion, this study demonstrated that alongside CS and SS classification, the SS/CS mixed subtype can also be observed. It was emphasized that the SS/CS mixed subtype, which exhibited a small amount of PAS positivity, might encounter CIS structure in the SS type in its early stages. In addition to PAS staining for diagnosis, PLAP immunohistochemical staining is recommended. It should be noted that spermatocytic seminomas observed in an intratubular pattern generally tend to transform into a diffuse form.

Conflict of Interest

The authors stated that they did not have anyreal, potential or perceived conflict of interest.

Ethical Approval

This study is not subject to HADYEK permission in accordance with Article 8 (k) of the "Regulation on Working

Procedures and Principles of Animal Experiments Ethics Committees".

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Similarity Rate

We declare that the similarity rate of the article is 7% as stated in the report uploaded to the system.

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Author Contributions

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Design: GYT
Control/Supervision: AST, GYT
Data Collection and / or Processing: GYT, AST
Analysis and / or Interpretation: GYT, AST
Literature Review: GYT, AST
Writing the Article: GYT
Critical Review: AST

References

Agnew DW, MacLachlan NJ, 2017: Tumors of the genital systems. In Meuten DJ, editor. Tumors in Domestic Animals, 5th ed. Wiley, p 689-722.

Akin I, Avci H, Gulaydin A, Belge A, Yaygingul R, 2013: Bilateral malignant seminoma in two dogs. *Kafkas Univ Vet Fak Derg,* 19 (Suppl-A): A233-A236.

Bush JM, Gardiner DW, Palmer JS, Rajpert-De Meyts E, Veeramachaneni DNR, 2011: Testicular germ cell tumours in dogs are predominantly of spermatocytic seminoma type and are frequently associated with somatic cell tumours. *Int J Androl*, 34(4pt2), e288-e295.

Grieco V, Riccardi E, Greppi GF, Teruzzi F, Iermano V, Finazzi M, 2008: Canine testicular tumours: a study on 232 dogs. *J Comp Path*, 138 (2-3), 86-89.

Grieco V, Riccardi E, Rondena M, Ciampi V, Finazzi M, 2007: Classical and spermatocytic seminoma in the dog: histochemical and immunohistochemical findings. *J Comp Pathol*, 137 (1), 41- 46.

Hernández-Jardón N, Rojas-Castañeda JC, Landero-Huerta D, Reyes-Cruz E, Reynoso-Robles R, Juárez-Mosqueda MDL, Medrano A, Reyes-Delgado F, Vigueras-Villaseñor RM, 2022: Cryptorchidism: The dog as a study model. *Front Vet Sci*, 9, 935307.

Kennedy PC, Cullen JM, Edwards JF, Goldschmidt MH, Larsen S, Munson L. 133 and Nielsen S, 1998: Histological Classification of genital tumour in domestic animals, 134.

Kristensen P, Andersen A, Irgens LM, Bye AS, Vagstad N, 1996: Testicular cancer and parental use of fertilizers in agriculture. *Cancer Epidemiol Biomarkers Prev*, 5, 3-9.

Lamb JC, Mark TA, Gladen BC, Allen JW, Moore JA, 1981: Male fertility, sister chromatid exchange, and germ cell toxicity following exposure to mixtures of chlorinated phenoxy acids containing 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. *J Toxicol Environ Health*. *A Current Issues*, 8(5-6), 825-834.

Maiolino P, Restucci B, Papparella S, Paciello O, De Vico G, 2004: Correlation of nuclear morphometric features with animal and human World Health Organization International Histological Classifications of canine spontaneous seminomas. *Vet Pathol*, 41(6), 608-611.

Mostofy FK, Sesterhenn IA, 1998: Histological typing of testis tumours. In: World Health Organization International Histological Classification of Tumours, 2nd ed., World Health Organization, Geneva.

Nasciment HH, Santos AD, Prant AL, Lamego EC, Tondo LA, Flores MM, Fighera RA, Kommers GD, 2020: Testicular tumors in 190 dogs: Clinical, macroscopic and histopathological aspects. *Pesquisa Vet Bras*, 40, 525-535.

Nielsen S, 1998: Histological classifications of tumors of the genital system of domestic animals. In: World Health Organization International Histological Classification of Tumors of Domestic Animals, Vol. IV, Armed Forces Institute of Pathology, Washington D.C., p. 17e18.

Özsoy ŞY, Kutsal O, 2007: Bir köpekte malign seminom. *Ankara Üniv Vet Fak Derg*, 54 (1), 55-59.

Skakkebaek N, Berthelsen J, Giwercman, A, Müller J, 1987: Carcinoma-in-situ of the testis: possible origin from gonocytes and precursor of all types of germ cell tumours except spermatocytoma. *Int J Androl*, 10 (1), 19-28.

Yumuşak N, Çalışkan M, Kutsal O, 2014: Bilaterally diffuse malignant seminoma in a dog. *Ankara Üniv Vet Fak Derg*, 61 (2), 151-152.

HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Staphylococcus aureus, Escherichia coli, *Pseudomonas aeruginosa* ve farklı Candida standart kökenlerine karşı Kına'nın antimikrobiyal aktivitesinin tespiti: Bir in vitro çalışma

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Özet: Lythraceae familyasının Lawsonia cinsindeki tek tür olan *Lawsonia inermis* (kına ağacı) 2-6 m yüksekliğinde çiçekli bir bitkidir. *Lawsonia inermis* (kına), dünya çapında cilt ve saç boyamak için yaygın olarak kullanılan boya kınanın doğal kaynağıdır. Kına içeriğinde bulunan farklı biyoaktif bileşenlerin, antimikrobiyal özellikleri olduğu bilinmektedir. Çalışmamızda, ticari olarak ülkemizde satılan kına tozunun (su bazlı ekstraktının) *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 ve *Pseudomonas aeruginosa* ATCC 27853 standart kökenlerine karşı antibakteriyel ve *Candida albicans* ATCC 10231, *C. tropicalis* ATCC 750, *C. krusei* ATCC 6258 ve *C. glabrata* ATCC 2001 standart kökenlerine karşı antifungal aktivitesinin kontrol edilmesi amaçlanmıştır.

Staphylococcus aureus ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 10231, *C. tropicalis* ATCC 750, *C. krusei* ATCC 6258 ve *C. glabrata* ATCC 2001 standart kökenleri çalışmamızda kullanılmıştır. 20 µg/mL ile 0.3125 µg/mL arasında kına tozunun su bazlı ekstraktları hazırlanmış ve mikrodilüsyon metodu kullanılarak antimikrobiyal aktivite belirlenmiştir. Her kuyucuktaki bakteri ve mantar üremesi, 600 nm'de Epoch spektrofotometre ile ölçülmüş ve pozitif kontrole göre baskılanma oranları saptanmıştır.

Tüm standart kökenlerine karşı hiçbir konsantrasyonda %100 baskılama olmadığı saptandı. *S.aureus, E. coli*'ye ve *P.aeruginosa*'ya karşı 20 µg/mL kına konsantrasyonunda sırasıyla %64, %55 ve %49 seviyesinde baskılama tespit edildi. *C. albicans, C. tropicalis, C. krusei* ve *C. glabrata* karşı 20 µg/mL kına konsantrasyonunda sırasıyla %92, %64, %56 ve %42 seviyesinde baskılama saptandı.

Sonuç olarak, çalışmamız verileri su bazlı kına ekstraktının antibakteriyel etkisinin *S. aureus, E.coli* ve *P. aeruginosa*'ya karşı çok iyi olmadığını yüksek konsantrasyonlarda bu etkinin gözlemlenebileceğini bize göstermiştir. Bunun yanında, kınanın farklı *Candida* türleri açısından da, özellikle *C. albicans*'a karşı kullanılabileceği ve diğer antifungallerle birlikte kullanımlarının değerlendirilmesi kanaati oluşmuştur.

Anahtar Kelimeler: *Antimikrobiyal etkinlik, Candida spp., E. coli, Kına, P. aeruginosa, S. aureus.*

Detection of antimicrobial activity of henna against standard strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and different *Candida* standard strains: An in vitro study

Abstract: *Lawsonia inermis* (henna tree), the only species in the Lawsonia genus of the Lythraceae family, is a flowering plant with a height of 2-6 m. *Lawsonia inermis* (henna) is the natural source of the dye henna, which is widely used worldwide to dye skin and hair. Different bioactive components in henna are known to have antibacterial properties. The aim of this study was to control the antibacterial activity of henna powder (water-based extract) commercially sold in our country against *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 and to control the antifungal activity of henna powder against *Candida albicans* ATCC 10231, *C. tropicalis* ATCC 750, *C. krusei* ATCC 6258 and *C. glabrata* ATCC 2001 standard strains.

Standard strains of *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 10231, *C. tropicalis* ATCC 750, *C. krusei* ATCC 6258 and *C. glabrata* ATCC 2001 were used in our study. Water-based extracts of henna powder between 20 µg/mL and 0.3125 µg/mL were prepared and antimicrobial activity was determined using the microdilution method. Bacterial and fungal growth in each well was measured by Epoch spectrophotometer at 600 nm and suppression rates were determined compared to the positive control.

No 100% suppression was detected at any concentration against the standard strains. Against *S. aureus, E. coli* and *P. aeruginosa*, suppression was detected at the levels of 64%, 55% and 49% at 20 µg/mL henna concentration, respectively. Against *C. albicans, C. tropicalis*, C. *krusei* and *C. glabrata*, suppression of 20 µg/mL henna concentration was 92%, 64%, 56% and 42%, respectively.

In conclusion, the data of our study showed us that the antibacterial effect of water-based henna extract is not very good against *S. aureus, E. coli* and *P. aeruginosa*, but this effect can be observed at high concentrations. In addition, it has been concluded that henna can be used against different *Candida* species, especially against *C. albicans*, and its use together with other antifungals has been evaluated.

Keywords*: Antimicrobial effectivity, Candida spp., E. coli, Henna, P. aeruginosa, S. aureus.*

Giriş

Bitkisel ilaçlar, değerli bir antimikrobiyal etken kaynağı olabilecekleri için dünya çapında çok popülerdir (Mardani ve ark., 2018). *Lawsonia inermis*, syn. L. alba (kına) bitkisi 2-6 m yüksekliğinde çiçekli bir bitkidir ve Lythraceae familyasının Lawsonia cinsindeki tek türdür. Bu bitkinin geleneksel tıpta yüzyıllardır kullanıldığı bilinmektedir (Al-Rubiay ve ark., 2008). Lawsonia inermis (kına), dünya çapında cilt ve saç boyamak için yaygın olarak kullanılan boya kınanın doğal kaynağıdır. Yapraklar geleneksel olarak kına boyasının hazırlanmasında kullanılır (Elansary ve ark., 2020). Ülkemizde, kına; hem kozmetik alanda, hemde farmakolojik olarak kullanılmaktadır (Malas ve Ekici, 2022). Kına içeriğinde farklı biyoaktif bileşenler bulunmaktadır ve ana kimyasal bileşenlerinin, 2-dihidroksinaftokinon (lawson), mannit, müsilaj, gallik asit ve tanik asit gibi kimyasallar olduğu bilinmektedir (Akshaykranth ve ark., 2021; Al-Rubiay ve ark., 2008). Bu bitki, ayrıca linalool alfa terpineol, etherfenilvinil A 1,3-indandion, eugenol,2-hidroksi-1, 4-naptokinon, oksiran-tetradesil, heksadekanoik asit gibi kimyasallara da sahiptir ve bu bileşiklerinde özellikle hidroksilleri stabilize edici özellikleri bilinmektedir (Akshaykranth ve ark., 2021). Tüm bu bileşiklerin, bakteri hücre duvarında karbonhidratlar ve proteinlerle birleşerek antimikrobiyal aktiviteye neden olan çoklu serbest hidroksillere sahip olduğu bildirilmiştir. Biyoaktif özelliğinin yüksek protein bağlama kapasitesinden kaynaklandığı düşünülmektedir (Al-Rubiay ve ark., 2008; Elebeedy ve ark., 2022).

Kınanın; kabızlığın, yanıkların, basınç ülserlerinin veya baş ağrısının tedavisi gibi farklı durumlar için halk geleneği olarak halk arasında yaygın bir kullanıma sahip olduğu bilinmektedir (Mutluoğlu ve Uzun, 2009; Rafiei ve ark., 2019). Çalışmalar kınanın içeriğinde bulunan kimsayallar aracılığıyla antibakteriyel, antifungal, analjezik ve antienflamatuar etkileri olduğunu göstermiştir (Kayıkcı ve ark 2020; Mutluoğlu ve Uzun, 2009; Rahmoun ve ark., 2013). Geleneksel tıpta kullanılan bitkisel ilaçlar, günümüzde mevcut ticari antibiyotiklerin artan direnç ve toksisite sorununun üstesinden gelmeye yardımcı olabilecek kemoterapi için potansiyel yeni ilaçların geliştirilme potansiyelleri nedeniyle ilgi çekicidir. Şifalı bitkilerin kullanım amaçlarını doğrulamak, içeriğinin izolasyonu ve karakterizasyonu bu potansiyelinin ortaya çıkartılabilmesi açısından çok önemlidir (Rahmoun ve ark. 2010; Spellberg ve ark., 2008).

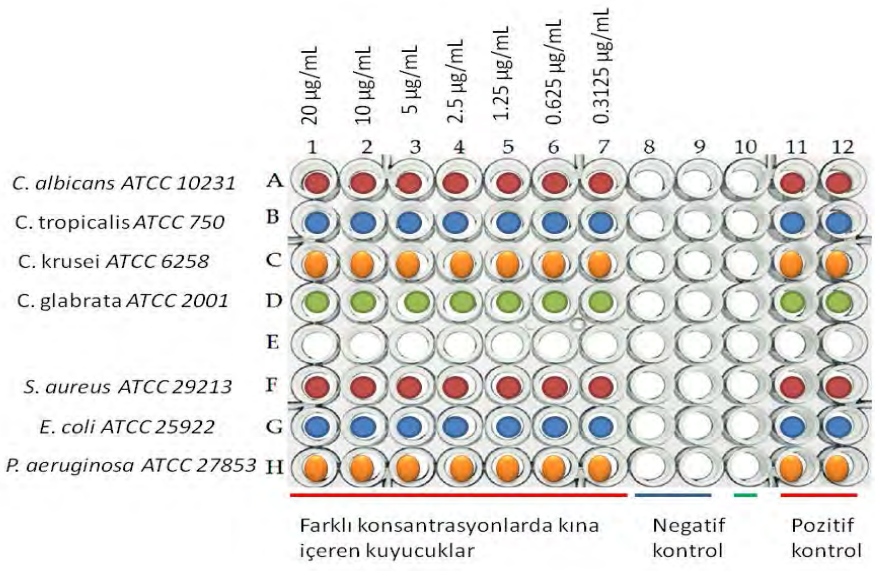
Çalışmamızda, ticari olarak ülkemizde satılan kına tozunun (su bazlı ekstraktının) *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 ve *Pseudomonas aeruginosa* ATCC 27853 standart kökenlerine karşı antibakteriyel aktivitesinin ve *Candida albicans* ATCC 10231, *C. tropicalis* ATCC 750, *C. krusei* ATCC 6258 ve *C. glabrata* ATCC 2001 standart kökenlerine karşı antifungal aktivitesinin kontrol edilmesi amaçlanmıştır.

Materyal ve Metod

Standart bakteriyel kökenler: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 ve *Pseudomonas aeruginosa* ATCC 27853 standart kökenleri çalışmamızda kullanılmıştır. Bu çalışma "Hayvan Deneyleri Etik Kurullarının Çalışma Usul ve Esaslarına Dair Yönetmelik" Madde 8 (k) gereği HADYEK iznine tabi değildir''.

Standart Candida kökenleri: Çalışmamızda Candida albicans American tip kültür kolleksiyonu (ATCC) 10231, *C. tropicalis* ATCC 750, *C. krusei* ATCC 6258 ve *C. glabrata* ATCC 2001 standart mantar kökenleri kullanılmıştır.

Kına tozunun su bazlı ekstraktının oluşturulması: Su bazlı ekstrakt hazırlamak için, 25 g kına tozu 250 mL steril distile su ile 12 saat karıştırılmasıyla hazırlandı. Bu karışım filtre kağıdı (Wattman No. 185) ile süzüldü. Kullanımı sırasında miktarların ayarlanabilmesi için evaporatör kullanılarak 50°C'de konsantre edildi (Al-Rubiay ve ark., 2008).



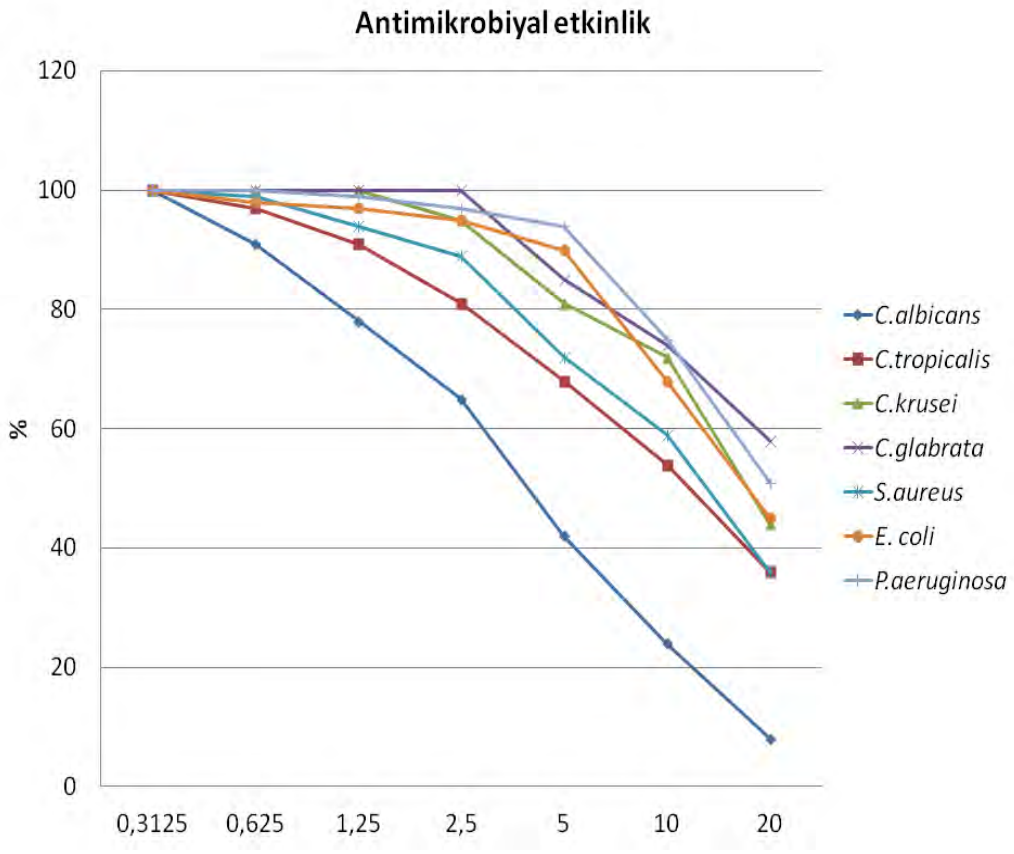
Şekil 1. Kınanın antimikrobiyal etkinliğini test etmek amaçlı 96 kuyucuklu plakda mikrodilüsyon metodunun uygulama şekli

Kına tozunun su bazlı ekstraktına karşı antimikrobiyal aktivitesinin belirlenmesi: Mikrodilüsyon metodu antimikrobiyal aktivitenin belirlenmesi için CLSI yönergeleri uygulandı. Bunun için 96 kuyucuklu plakalardaki 1. sıra kuyucuklarına kına tozunun final konsantrasyonu 20 µg/mL olacak 180 µl eklendi. 2, 3, 4, 5, 6 ve 7. sıra kuyucuklarında ise sırasıyla, 10 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL, 0.625 µg/mL ve 0.3125 µg/mL final konsantrasyonlarını oluşturmak amaclı 90 µl BHI sıvı besiyeri eklendi. 1.sıra kuyucuğundan 2'ye, 2'den 3'e olacak şekilde kuyucuklarda dilüsyonlar gerçekleştirildi. Bir gece 37 °C'da Brain Heart Infusion Broth (BHI) sıvı besiyeri içerisinde inkübasyona bırakılan standart mantar veya bakteri kültürlerinden spektrofotometrede OD ölçümleri yapılarak 0.5 McFarland tubidite standartında 1-6 sıra aralığındaki tüm kuyucuklara 10 µl standart *Candida* veya standart bakteri kökenleri ilave edildi. 8 ve 9. sıraya negatif kontrol amaçlı kına ilave edilmiş ve mantar veya bakteri içermeyen BHI sıvı besiyeri 10. sıraya kına ilave edilmemiş ve mantar veya bakteri içermeyen BHI sıvı besiyeri, 11. ve 12. sıraya ise pozitif kontrol amaçlı mantar veya bakteri içeren kına içermeyen, BHI sıvı besiyeri ilave edildi (Şekil 1). Kuyucuklarda toplam 100 µl hacim ile çalışıldı. Bir gece 37 °C'da inkübasyona bırakılan 96 kuyucuklu plakalarda, her kuyucuktaki mantar veya bakteri üremesi, 600 nm'de Epoch spektrofotometresi (Biotek, Almanya) ile ölçüldü. Kına içermeyen pozitif kontrol besiyerleri karşılaştırma için kullanıldı (Özdal Zincir ve ark. 2021; Unlu ve ark.,2021). Mikroorganizma ve kına içeren mikrokuyucuklardan elde edilen spektrofotometrik okuma değerlerin negatif kontrol kuyucuğundan elde edilen okuma değerinden düşük olmaması ve pozitif kontrol kuyucuğundan elde edilen okuma değerinden yüksek olmaması değerlendirme kriterleri olarak dikkate alındı. Her bir kuyucuk için 10 tekrar yapılarak işlemler tekrar edildi.

Bulgular

Çalışmamızda, ticari olarak ülkemizde satılan kına tozunun (su bazlı ekstraktının) *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 ve *Pseudomonas aeruginosa* ATCC 27853 standart kökenlerine karşı antibakteriyel aktivitesini kontrol ettiğimizde, hiçbir kökende, hiçbir konsantrasyonda %100 baskılama olmadığı saptandı. Bu nedenle incelenen konsantrasyonlarda bu kökenler için minimal inhibitör konsantrasyon (MIC) ve minimal bakterisidal konsantrsayon (MBC) değeri saptanamadı.

S. aureus'a karşı 20 µg/mL kına konsantrasyonunu %64 seviyesinde üremede baskılama sağlarken, 10 µg/mL konsantrasyonda bu baskılama seviyesi %41 seviyesinde kaldığı tespit edildi. *E. coli*'ye karşı 20 µg/mL kına konsantrasyonunun %55, 10 µg/mL kına konsantrasyonun ise %32 baskılama sağlayabildiği bulundu. *P. aeruginosa*'ya karşı ise 20 µg/mL kına konsantrasyonunun %49,10 µg/mL kına konsantrasyonun ise ancak %25 üremeyi baskılama potansiyeli olduğu tespit edildi. Gram pozitif *S. aureus*'a karşı antibakteriyel etkisinin, Gram negatif *E. coli* ve *P.aeruginosa*'ya göre daha iyi olduğu bulundu (Şekil 2).



Şekil 2. Farklı kına konsantrasyonlarının standart *S. aureus, E. coli, P. aeruginosa* ve farklı *Candida* spp. karşı baskılama oranları.

Kına tozunun (su bazlı ekstraktının) *Candida albicans* ATCC 10231, *C. tropicalis* ATCC 750, *C. krusei* ATCC 6258 ve *C. glabrata* ATCC 2001 standart kökenleri gibi farklı *Candida* türlerine karşı antifungal aktivitesini kontrol ettiğimizde, hiçbir kökende, hiçbir konsantrasyonda %100 baskılama tespit edilmediği bulundu. Bu nedenle incelenen konsantrasyonlarda bu kökenler için minimal inhibitör konsantrasyon (MIC) ve minimal bakterisidal konsantrsayon (MBC) değeri hesaplanmadı.

C.albicans'a karşı 20 µg/mL kına konsantrasyonunu %92 seviyesinde üremede baskılama sağlarken, 10 µg/mL konsantrasyonda bu baskılama seviyesi %76 seviyesinde kaldığı tespit edildi. *C. tropicalis*'e karşı 20 µg/mL kına konsantrasyonunun %64, 10 µg/mL kına konsantrasyonun ise %46 baskılama sağlayabildiği bulundu. *C. krusei*'ye karşı ise 20 µg/mL kına konsantrasyonunun %56, 10 µg/mL kına konsantrasyonun ise ancak %38 üremeyi baskılama potansiyeli olduğu tespit edildi. *C. glabrata*'da ise 20 µg/mL kına konsantrasyonunun %42, 10 µg/mL kına konsantrasyonun ise ancak %26 üremeyi baskıladığı bulundu. *Candida* türleri arasında en yüksek etkinliği 20 µg/mL konsantrasyonda *C. albicans* üstüne tespit edildi (Şekil 2).

Tartışma ve Sonuç

Lawsonia inermis veya kına ağacı, Asya, Amerika ve Afrika'nın çeşitli tropikal bölgelerinde yaygın olarak yetiştirilmektedir ve bu bitkinin geleneksel tıpta yüzyıllardır kullanıldığı bilinmektedir (Al-Rubiay ve ark., 2008). Bizde bu nedenle çalışmamızda, kınanın antibakteriyel özelliğine odaklanarak, *S. aureus, E. coli* ve *P. aeruginosa* gibi önemli patojen bakterilere karşı antibakteriyel etkinliğini ölçmeye ve farklı *Candida* türlerine karşı antifungal etkinliğini tespit etmeye odaklandık.

Al-Rubiay ve ark. (2008) kınanın farklı kına ekstraktlarının antimikrobiyal etkinliğini incelediklerini ve su ekstraktının herhangi bir etkinliğinin olmadığını bildirmişlerdir. Bizde çalışmamızda kınanın su ekstraktına karşı bu bakterilerde MIC ve MBC oluşturacak doza çıkamadığımı tespit ettik. Habbal ve ark. (2011) çalışmalarında kına ekstraktının *P. aeruginosa*'ya karşı etkin olduğunu bildirmişlerdir. Al-Rubiay ve ark. (2008) ve Habbal ve ark.'nın (2021) çalışmaları incelendiğinde alkol bazlı kına ekstraktlarında antibakteriyel aktivite saptadıkları görülmektedir. Fakat etanol gibi alkollerin bakterisidal etkinlikleri bilinmektedir. Sauerbrei (2021) ve bu çalışmalarda alkol bazlı kına ekstraktının etkisi olarak bildirdikleri etkinin etanolün bakterisidal etkisi olabileceğini bize düşündürmüştür. Usman ve Rabiu (2018), çalışmalarında *S. aureus*'a karşı duyarlılığın 1000 µg/mL olduğunu bildirmişlerdir. Bu doz bizim araştırdığımız dozun 100 katı yüksek olması dolayısıyla, sonucun normal olduğu düşünülmüştür. Fakat bu kadar yüksek dozların (fareler üstüne yapılan deneylerde 500 mg/kg) şiddetli klinik tablolara neden olabileceği hatta ölüme neden olabileceği akılda tutulmalıdır (SCCS, 2013). Mirjalili ve ark. (2014)'da kına'nın tekstil boyalarına mordant olarak katılmasının *E.coli* ve *S. aureus* gibi bakterilere karşı antibakteriyel etkinliği arttırdığını saptamışlardır. Çalışmalarında özellikle kına'nın, ferrik sülfat, kuprik sülfat ve potasyum alüminyum sülfat gibi antibakteriyel özellik gösteren kimyasallar varlığında daha iyi performans gösterdiğini belirtmişlerdir (Mirjalili ve ark., 2014). Bu da özellikle kınanın diğer antibakteriyel etkili ajanlarla birlikte kendi etkisininde sinerjik olarak artabileceğini bize düşündürmüştür. Ibrahim ve ark. (2021) kına ekstraktının *S. auerus* başta olmak üzere gram pozitif bakterilere karşı iyi olduğunu ve ilaç olarak kullanabileceğini bildirmiştir. Fakat diğer alkol bazlı kına ekstraktı çalışmalarına benzer şekilde (Al-Rubiay ve ark. 2008; Habbal ve ark., 2011) bu çalışmada da alkol bazlı ekstrakt kullanılmış ve sonuçlar bunun üstünden yorumlanmıştır (Ibrahim ve ark., 2021). Verilerimizin bu nedenle benzer olmadığı, su bazlı ekstraktın etkinliğinin bu nedenle düşük olduğu düşünülmüştür.

Yiğit (2017) çalışmasında, farklı klinik izolatlardan elde ettikleri farklı *Candida* türlerinin kına ile etkileşimini araştırmışlar ve kınanın antifungal özelliğini incelemişlerdir. Zon çaplarını incelediklerinde *C. albicans*'ların %25.9'unun, *C. krusei*'lerin %20'sinin, *C. tropicalis*'lerin %15.3'ünün ve *C. glabrata* kökenlerinin %35.2'sinin direnç gösterdiğini bildirmişlerdir. En yüksek antifungal aktivitenin *C. albicans*'a karşı olduğunu tespit etmişlerdir, bunu *C. glabrata, C. tropicalis* ve *C. krusei*'nin takip ettiğini bildirmişlerdir (Yiğit, 2017). Bizim çalışmamızda standart kökenler kullanılmasına karşın *C. albicans*'a karşı en etkin olduğununun tespiti yönüyle sonuçlarımız benzerdir. *C. albicans*'ın üremesine karşı kına bileşiklerinin özellikle toksisite ve farmakokinetik özellikleri açısından da önemli etkileri olabildiği bilinmektedir (Nawasrah ve ark., 2016). Benzer şekilde *C. albicans*'a karşı kınanın su ve etanolik ekstraktının etkili olduğu bildirilmiştir (Yarako, 2015). Suleiman ve Mohamed. (2014) 10 mg/mL'lik etanolik kına ekstraktının *C. albicans*'a karşı 17 mm inhibisyon zonu geliştirdiği gösterilmiştir. Buna karşın petrolyum ekstraktı oluşturulduğunda bu inhibisyon zonu 22.7 mm'e çıkmıştır. Bu veri çalışmalardaki kına ekstraktı için kullanılan maddeninde antifungal aktiviteye etki ettiğini göstermektedir. Bizde çalışmamızda su kullanarak bu etkiyi en aza indirmeye çalıştık.

Candida türleri dışında farklı mantarlar üstüne de kına ile çalışmalar yapıldığı tespit edilmektedir. Dermatofitler, *Fusarium*, *Aspergillus* gibi farklı mantar türleri üstüne de kınanın etkinliği denenmiş ve farklı miktarda etkinlik bilgileri tespit edilmiştir (Gozubuyuk ve ark., 2014; Rahmoun ve ark., 2013).

Deney hayvan modelinde vajinal kandidiyazise karşı İran kınasının etkili olduğu ve vajinal krem olarak *C. albicans*'a karşı kullanılan İran kınasının %4'ünün etkili olduğu ve antifungal klotrimazol ile benzer etkinlik düzeyine sahip olduğunu bildirmişlerdir (Yaralizadeh ve ark., 2018). Gad ve ark. (2021) beyaz kına ve doğal kınanın resinlere katılarak *C. albicans*'ın adhezyonunu engelleyebileceğini bildirmişlerdir.

Çalışmamızın in-vitro tasarlanması, standart kökenlerin kullanılması ve tek tip kına tozunun kullanılmış olması çalışmamızın kısıtlamalarıdır. Fakat elde ettiğimiz veriler hem önemli bakteriyel patojenlere karşı kınanın antibakteriyel

etkinliğinin gösterilmiş olması, hemde farklı *Candida* türlerine karşı kınanın antifungal etkinliğini gösteren çalışma olması nedeniyle değerlidir.

Sonuç olarak, çalışmamız verileri su bazlı kına ekstraktının antibakteriyel etkisinin *S. aureus*, *E.coli* ve *P. aeruginosa*'ya karşı çok iyi olmadığını yüksek konsantrasyonlarda bu etkinin gözlemlenebileceğini bize göstermiştir. Tıbbi kullanımında antibakteriyel etkinlik için kullanılması gereken dozların yüksek olabileceği ve bunun farklı yan etkilere neden olabileceği akılda bulundurulmalıdır. Ayrıca çalışmamız verileri kınanın farklı *Candida* türleri açısından *C. albicans*'a karşı en etkin olabildiğini ve diğer antifungallerle birlikte kullanımlarının değerlendirilmesi gerektiğini bize düşündürmüştür.

Çıkar çatışması

Yazarlar bu yazı için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan etmişlerdir.

Etik kurul

Hayvan Deneyleri Etik Kurullarının Çalışma Usul ve Esaslarına Dair Yönetmelik" Madde 8 (k) gereği HADYEK iznine tabi değildir. Ayrıca yazarlar Araştırma ve Yayın Etiğine uyulduğunu beyan etmişlerdir.

Benzerlik Oranı

Makalenin benzerlik oranının sisteme yüklenen raporda belirtildiği gibi % 14 olduğunu beyan ederiz.

Yazar Katkıları

Fikir/Kavram: HD
Tasarım: MD
Denetleme/Danışmanlık: MD, FYZ
Veri Toplama ve/veya İşleme: HD, AY
Analiz ve/veya Yorum: AY
Kaynak Taraması: AY, MD
Makalenin Yazımı: MD, HD
Eleştirel İnceleme: AY, FYZ

Kaynaklar

Akshaykranth A, Jayarambabu N, Tumu VR, 2021: Comparative Study on Antibacterial Activity of MgO Nanoparticles Synthesized from Lawsonia inermis Leaves Extract and Chemical Methods. *J Inorg Organomet Polym,* 31, 2393–2400

Al-Rubiay KK, Jaber NN, Al-Mhaawe BH, Alrubaiy LK, 2008: Antimicrobial efficacy of henna extracts. *Oman Med J*, 23 (4), 253-256.

Elansary HO, Szopa A, Kubica P, Ekiert H, A Al-Mana F, Al-Yafrsi MA, 2020: Antioxidant and Biological Activities of Acacia saligna and Lawsonia inermiş. *Natl Pop Plants*. 17 (7), 908.

Elebeedy D, Ghanem A, El-Sayed M, Fayad E, Abu Ali OA, Alyamani A, Sayed Abdelgeliel A, 2022: Synergistic Antimicrobial Effect of Lactiplantibacillus plantarum and Lawsonia inermis Against Staphylococcus aureus. *Infect Drug Resist*. 19 (15), 545-554.

Gad M, Al-suny M, Al-shayeb A, Al-namsy R, Al-naser Z, Khan S, 2021: The in-vitro effects of white henna addition on the *cCandida albicans* adhesion and physical properties of denture base resin. *Eur Oral Res*, 55 (2), 86-93.

Gozubuyuk GS, Aktas E, Yigit N, 2014: An ancient plant Lawsonia inermis (henna): determination of in vitro antifungal activity against dermatophytes species. *J Mycol Med*, 24 (4), 313-318.

Habbal O, Hasson SS, El-Hag AH, Al-Mahrooqi Z, Al-Hashmi N, Al-Bimani Z, Al-Balushi MS, Al-Jabri AA, 2011: Antibacterial activity of Lawsonia inermis Linn (Henna) against *Pseudomonas aeruginosa*. *Asian Pac J Trop Biomed,* 1 (3),173-176.

Ibrahim SMS, Rasool CS, Al-Asady AA, 2021: Antimicrobial activity of crude henna extract against Gram-positive bacteria. *Iraq Medical Journal,* 5 (3), 89–93.

Kayikci EE, Can G, Sen F, Saip P, 2020: Application in the Prevention of Capecitabine-Induced Hand-Foot Syndrome in Breast and Colorectal Cancer Patients. *J Nurs. Oct*, 26 28(3), 299-311.

Malas B, Ekici, H, 2022: Lawsonia inermis (Kına) ve Farmakolojik Özellikleri. *Türk Bil Derl Derg*. 15 (1), 121–131.

Mardani M, Badiee P, Gharibnavaz M, Jassebi A, Jafarian H, Ghassemi F, 2018: Comparison of anti-*Candida* activities of the ancient plants Lawsonia inermis and Ziziphus spina christi with antifungal drugs in *Candida* species isolated from oral cavity. *J Conserv Dent*. 21 (4), 359-362.

Mirjalili M, Karimi L, Paydar H, Chizarifar G, 2014: Effect of henna natural dye on antibacterial properties of dyed nylon fabric with various mordants. *Iran JOC*, 6 (4), 1389-1395.

Mutluoğlu M, Uzun G, 2009: Can henna prevent ulceration in diabetic feet at high risk? *Exp Diabetes Res*, 107496.

Nawasrah A, AlNimr A, Ali AA, 2016: Antifungal Effect of Henna against *Candida albicans* Adhered to Acrylic Resin as a Possible Method for Prevention of Denture Stomatitis. *Int J Environ Res Public Health*, 23 13 (5), 520.

Özdal Zincir Ö, Özdal U, Ünlü Ö, Demirci M, Katiboğlu AB, Egil E, Altan Şallı G, 2022: Synergistic effect of thymoquinone and nystatin in the treatment of oral candidiasis; an in vitro study. *Odontology*, 110 (2), 330-337.

Rafiei Z, Mazaheri M, Eghbali-Babadi M, Yazdannik A, 2019: The Effect of Henna (Lawsonia Inermis) on Preventing the Development of Pressure Ulcer Grade One in Intensive Care Unit Patients. *Int J Prev Med*. 15, 10-26.

Rahmoun MN, Benabdallah M, Villemin D, Boucherit K, Mostefa-Kara B, Ziani-Cherif C, Choukchou-Braham N, 2010: Antimicrobial screening of the Algerian Lawsonia inermis (henna). *Der Pharma Chemica*. 2 (6), 320-326.

Rahmoun N, Boucherit-Otmani Z, Boucherit K, Benabdallah M, Choukchou-Braham N, 2013: Antifungal activity of the Algerian Lawsonia inermis (henna). *Pharm Biol*. 51 (1), 131-135.

Sauerbrei A, 2020: Bactericidal and virucidal activity of ethanol and povidone-iodine. *Microbiologyopen*. 9 (9), 1097.

SCCS (Scientific Committee on Consumer Safety), Opinion on Lawsonia inermis (henna), 19 September 2013.

Spellberg B, Guidos R, Gilbert D, Bradley J, Boucher HW, Scheld WM, Bartlett JG, Edwards J Jr. 2008: Infectious Diseases Society of America. The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. *Clin Infect Dis*, 15 46 (2), 155-164.

Suleiman EA, Mohamed EA, 2014: In Vitro Activity of Lawsonia inermis (Henna) on Some Pathogenic Fungi. *Journal of Mycology*, 375932.

Unlu O, Bingul ES, Kesici S, Demirci M, 2021: Investigating antimicrobial features and drug interactions of sedoanalgesics

in intensive care unit: an experimental study. *ADMET DMPK,* 6 9 (3), 219-226.

Usman RA, Rabiu U, 2018: Antimicrobial activity of Lawsonia inermis (henna) extracts. Bayero J of Pure and App Sci, 11,1.

Yarako AA, Salim R, Mohamed Z, Mohamad I, 2015. Antifungal Activity of Malaysian Henna Leaves Extracts on Pathogenic Fungi of Otomycosis. *Int Med J*, 22 (5), 389 – 391.

Yaralizadeh M, Abedi P, Namjoyan F, Fatahinia M, Nezamivand Chegini S, 2018: A comparison of the effects of Lawsonia inermis (Iranian henna) and clotrimazole on Candida albicans in rats. *J Mycol Med*. 28 (3), 419-423.

Yiğit D, 2017: Antifungal Activity Of Lawsonia Inermis L. (Henna) Against Clinical *Candida* Isolates. *Erzincan Unv J of Sci and Tech*, 10 (2), 196-202.

HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

The Effect of Season on the Performance, Health, and Welfare of Broilers*

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Abstract: The research was carried out on 80 broiler flocks (Ross 308) raised during the winter, spring, summer, and autumn seasons in commercial enterprises in Uşak City Center and its districts engaged in contract farming. Broiler flocks were examined weekly during the 0-42 day growth period for body weight, weight gain, feed consumption, feed conversion ratio, mortality and EPEF. The 42-day-old broiler was scored for FPD and HB. The season significantly impacted the broiler flock's performance and the birds' prevalence with FPD and HB under commercial conditions. Spring and autumn flocks showed higher performance than winter and summer flocks. The prevalence of birds with foot pad burn and hock burn was highest in winter flocks and lowest in summer flocks. These findings indicate that seasons can significantly impact broiler farms' economic performance and bird welfare. As a result, obtaining further information about the positive and negative effects of seasons on broilers can promote production and provide critical data to encourage innovative strategies for sustainable flock management and improve broiler industry compliance with animal welfare standards.

Keywords: *Broiler, Performance, Season, Welfare.*

Mevsimin Broiler Piliç Performansı ve Refahı Üzerine Etkisi

Özet: Araştırma, Uşak İl Merkezi ve ilçelerinde sözleşmeli üretim yapan ticari işletmelerde kış, ilkbahar, yaz ve sonbahar mevsimlerinde yetiştirilen 80 adet broiler piliç sürüsünde (Ross 308) yürütülmüştür. Broiler piliç sürüleri canlı ağırlık, canlı ağırlık artışı, yem tüketimi, yemden yararlanma oranı, ölüm oranı ve EPEF yönünden 0-42 günlük büyüme döneminde haftalık olarak incelenmiştir. Kırk iki günlük piliçler FPD ve HB yönünden skorlanmıştır. Mevsim ticari koşullardaki broiler sürülerin performansı ile FPD ve HB görülen piliç prevelansını önemli ölçüde etkilemiştir. İlkbahar ve Sonbahar sürüleri, Kış ve Yaz sürülerine göre daha yüksek performans göstermiştir. Ayak tabanı yanığı ve diz yanığı tespit edilen broiler piliçlerin prevalansının Kış sürülerinde en yüksek ve Yaz sürülerinde en düşük olduğu belirlenmiştir. Bu bulgular, mevsimlerin broiler işletmelerinin ekonomik performansı ile piliçlerin refahını önemli ölçüde etkileyebileceğini göstermektedir. Sonuç olarak, mevsimlerin broiler piliçlerin üzerindeki olumlu ve olumsuz etkileri hakkında daha fazla bilgi edinmenin, üretimi teşvik edebileceği ve sürdürülebilir sürü yönetimi için yenilikçi stratejileri teşvik etmek ve broiler endüstrisinin hayvan refahı standartlarına uyumunu geliştirmek için önemli veriler sağlayabileceği sonucuna varılmıştır.

Anahtar Kelimeler*: Broiler, Mevsim, Performans, Refah*

Introduction

Broiler hybrids, which have been subjected to high levels of selection for high productivity and rapid growth, have also become highly sensitive to environmental factors (Beg et al., 2011; Musilová et al., 2013). Therefore, optimizing the indoor conditions in poultry houses is crucial for maximum efficiency (Beg et al., 2011; De Jong et al., 2014; Federici et al., 2016). However, outdoor environmental conditions can make maintaining optimal conditions inside poultry houses challenging. Adverse indoor conditions have been shown to reduce broiler performance and threaten broiler health and welfare (De Jong et al., 2012; Guo et al., 2023).

Closed housing systems and indoor climate control systems have been introduced to optimize indoor conditions in poultry houses. However, they increase production costs and need to provide long-term and definitive solutions (Marcu et al., 2013; Nawaz et al., 2021). Additionally, the accelerating pace of global climate change highlights the importance of new insights that shed light on the present and near-future seasonal effects (Aksoy et al., 2021; De Jong et al., 2012). Further research is required to examine the effects of seasons on commercial broiler houses and broilers' performance and welfare, using actual field samples that cover the entire year. (Jones et al., 2005; Nawaz et al., 2021). This study investigates the effects of the year's seasons on the performance and welfare of broilers reared on commercial poultry farms.

Materials and Methods

Experimental Design, Flock Management and Production Performance

This study was conducted in commercial broiler farms in Uşak City Center and its districts engaged in contract farming. A total of 80 broiler flocks were used and raised during winter (December, January, February), spring (March, April, May), summer (June, July, August), and autumn (September, October, November) seasons in 2021 and 2022. The study utilized a cross-sectional sampling approach, randomly selecting 20 broiler farms (four flocks were used from each farm, reared in four different seasons) and poultry houses, focusing on the hybrid broiler type (Ross 308), poultry house and equipment characteristics, feeding program, and slaughter age (El-Tahawy et al., 2017; Federici et al., 2016; Kittelsen et al., 2017; Meluzzi et al., 2008).

All flocks were raised in littered houses equipped with automatic ventilation, heating, drinkers, and feeders for 0-42 days. The broiler management, feeding, vaccination, and health protection practices followed the Ross 308 management guide (Aviagen, 2020). The birds were provided with three different diets *ad libitum*: starter (23% CP and 3000 kcal ME/kg) from 1-10 days, grower (22% CP and 3100 kcal ME/kg) from 11-21 days, and finisher (20.5% CP and 3200 kcal ME/kg) from 21-42 days of age. On August 24, 2021, with reference number AKUHADYEK-88-21, this study was approved by the Local Animal Ethics Committee of Afyon Kocatepe University.

Litter and drinking water temperatures were measured using an infrared thermometer when chicks were placed in the house, and the number of chicks was recorded. A total of 50 chicks, randomly selected from five locations in the poultry house, were individually weighed using a digital precision scale precision 1g to determine average body weight (ABW) at one day of age (De Jong et al., 2012; Marcu et al., 2013). Using the same sampling method, the selected birds were individually weighed on days 7, 14, 21, 28, 35, and 42, and the average weekly body weights (ABW) for the groups were calculated. On the days the animals were weighed, the amount of feed remaining in the silos and troughs was subtracted from the total feed intake for the week to calculate the average weekly feed intake. On farms with silo weighing systems, the remaining feed was determined directly by weighing the silo. On the other farms, silo residues were collected, loaded onto lorries, and weighed. Weekly feed consumption was determined by subtracting the remaining feed at the end of the week from the total feed given that week. Average weekly feed intake per bird was calculated by dividing weekly feed intake by the number of birds per week. Mortality rate, total feed intake (TFI), and total weight gain (TWG= final body weight (g) - day one body weight (g)) were calculated on a weekly cumulative basis. Feed conversion ratio (FCR= cumulative feed intake (kg) /total weight gain (kg)) and livability (100 - mortality rate) were calculated weekly (Kryeziu et al., 2018; Kubiś et al., 2022; Meluzzi et al., 2008; Teyssier et al., 2022). The European Production Efficiency Factor was used to evaluate the broiler growth performance. It was calculated according to the formula proposed by Marcu et al. (2013) and Aviagen (2020) (EPEF= viability (%) x BW (kg)/age (d) x FCR (kg feed/kg gain) x100).

Data Collection and Lesion Scoring

In each flock, the number of dead birds was recorded daily using farm records. The mortality rates were calculated by dividing the cumulative number of dead birds each week by the initial chick numbers. The stocking density was calculated as m^2/bird (total number of chicks placed on the first day / total house area) and m^2/kg live weight (total live weight of flock's birds (the birds harvested on day 42 + the birds removed from the flock through thinning on days 33-36) / total house area).

Assessments of footpad and hock burn (FPD and HB) were carried out on broilers from 5 flocks in each season group when the broilers were 42 days old, taking into account the geographical distribution of broiler flocks and time constraints (only at 42 days of age). A total of 100 broilers were randomly selected, 25 birds each from 4 different areas along the length of the house, and both feet and knees of these birds were examined. According to the Welfare Quality® Poultry Welfare Assessment Protocol (Welfare Quality, 2009), each bird's foot pads and hock joint areas were observed and palpated for skin burns (contact dermatitis). The type, extent, and severity of the lesions were scored. Scoring was performed in five categories for the footpad and hock joint lesions of birds: no lesion (score:

0), superficial staining in an area less than 10% (score: 1), superficial staining in an area greater than 10% (score: 2), deep lesions and ulceration up to 50% (score: 3), and deep lesions and ulceration in an area greater than 50% (score: 4) (Freeman et al., 2020).

Statistical analysis: Statistical analysis was performed using a one-way analysis of variance to evaluate the differences in ABW, mortality rate, TWG, TFI, and FCR. The significance of differences between the seasonal groups was tested using the Duncan test. (Ural and Kılıç, 2013). Prevalences of FPD and HB scores were presented for each seasonal group. The statistical analyses for the studied parameters were performed using the SPSS 21 software package. The significance level was set at 0.05.

Results

The season significantly (P<0.05, P<0.001) influenced the ABW of broilers at 4, 5, and 6 weeks of age (Table 1).

Table 1. The average body weights and total weight gain of broilers in the season groups.

Weeks	Winter	Spring	Summer	Autumn	Total	P
ABW (g/bird)						
0	43.70 ± 0.76	44.45 ± 0.56	42.40 ± 0.94	44.15 ± 0.53	43.68±0.36	0.201^{-}
1	180.25 ±1.91	181.60 ±1.65	180.70 ±1.87	182.20 ±1.34	181.19 ±0.84	0.851^{-}
2	468.50 ± 6.96	476.25 ± 7.99	457.50 ± 7.03	466.15 ± 6.85	467.10±3.62	0.337^{-}
3	904.75 ± 10.13	918.75 ± 10.78	890.75 ± 11.56	915.95 ± 11.65	907.55±5.56	0.272^{-}
4	1475.00 ± 13.74^{a}	1476.00 ± 12.51^{a}	1404.00 ± 34.65^{b}	1479.75 ± 10.71^{a}	1458.69±10.61	0.028*
5	2021.80 ± 22.66^{bc}	2072.45 ± 23.51^{ab}	2003.05 ± 19.03^{c}	2085.80 ± 14.83^{a}	2045.78±10.68	0.013*
6	2602.60 ±24.18^{bc}	2660.70 ±31.24^{ab}	2535.65 ±29.53^{c}	2722.75 ±31.02^{a}	2630.43±16.27	0.000***
TWG (g/bird)						
1	136.55±1.65	137.15±1.70	138.30±1.97	138.05±1.33	137.51±.83	0.874^{-}
2	424.80±6.56	431.80±7.86	415.10±6.77	422.00±6.78	423.43±3.50	0.410^{-}
3	861.05±9.86	874.30±10.78	848.35±11.38	871.80±11.63	863.88±5.48	0.326^{-}
4	1431.30±13.54^{a}	1431.55±12.45^{a}	1361.60±34.25^{b}	1435.60±10.60^{a}	1415.01±10.47	0.030*
5	1978.10±22.37^{cb}	2028.00±23.54^{ab}	1960.65±18.83^{c}	2041.65±14.70^{a}	2002.10±10.59	0.015*
6	2558.90±23.77^{cb}	2616.25±31.20^{ab}	2493.25±29.22^{c}	2678.60±30.78^{a}	2586.75±16.14	0.000***

*:P<0.05, ***:P<0.00, $^{-}$: Non significant, ^{a,b,c} :Means of groups followed by different letters differ significantly at p<0.05, **ABW : A**verage Body Weight**, TWG:** Total Weight Gain

The highest ABW were observed during the winter, spring, and autumn seasons at four weeks, while at 5 and 6 weeks of age, the highest values were recorded in the spring and autumn seasons. The season had no significant effect on TWG, TFI, and FCR before 21 days. However, it significantly impacted TWG between 28-45 days (P<0.05, P<0.001), TFI between 35 and 42 days (P<0.001), and FCR in the 42 days (P<0.01) (Table 2).

Table 2. Total feed intake and feed conversion rate results by season in broilers,

Weeks	Winter	Spring	Summer	Autumn	Total	P
TFI (g/ bird)						
1	164.95 ±1.70	164.05±1.83	165.35±1.59	165.15±1.38	164.88±0.80	0.946^{-}
2	528.30±7.33	545.00±10.62	529.75±12.50	541.15±8.56	536.05±4.95	0.559^{-}
3	1202.50±17.26	1172.85±16.62	1146.95±14.06	1169.50±12.87	1172.95±7.83	0.093^{-}
4	2122.50±22.66	2103.75±16.88	2055.70±37.28	2087.75±19.94	2092.43±12.77	0.301^{-}
5	3193.00±38.40^{a}	3249.25±29.44^{a}	3068.20±32.44^{b}	3202.50±20.56^{a}	3178.24±16.92	0.001***
6	4322.24±53.62^{a}	4469.17±39.65^{a}	4114.89±66.49^{b}	4351.00±53.44^{a}	4314.33±30.18	0.000***
FCR (kg feed/kg gain)						
1	1.21±0.01	1.20±0.02	1.20±0.02	1.20±0.01	1.20±0.01	0.909^{-}
2	1.25±0.02	1.26±0.02	1.28±0.03	1.29±0.03	1.27±0.01	0.649^{-}
3	1.33±001	1.28±0.01	1.29±0.02	1.28±0.02	1.29±0.01	0.082^{-}
4	1.44±0.02	1.43±0.01	1.48±0.04	1.41±0.01	1.44±0.01	0.173^{-}
5	1.58±0.02	1.57±0.02	1.53±0.01	1.54±0.01	1.56±0.01	0.063^{-}
6	1.66±0.02^{ab}	1.68±0.02^{a}	1.62±0.02^{bc}	1.60±0.02^{c}	1.64±0.01	0.019**

**:P<0.01, ***:P<0.001, $^{-}$: Non significant, ^{a,b,c}:Means of groups followed by different letters differ significantly at p<0.05, T**FI**:Total Feed Intake, **FCR**: Feed Conversion Rate.

The highest TWG values were detected in Spring and Autumn. The winter, spring, and summer seasons showed the highest TFIs, while the winter and spring seasons exhibited the highest FCRs. Seasonal variation did not significantly affect the mortality rates of broiler flocks in the periods 0-42 days (Table 3). The differences in EPEF values between the seasonal groups were insignificant during the first five weeks. However, by week 6, the autumn flocks had significantly higher EPEF values (P<0.01) (Table 3).

Table 3. Mortality rates and EPEF by season in broilers.

Weeks	Winter	Spring	Summer	Autumn	Total	P
Mortality rate (%)						
1	1.47±0.12	1.07±0.13	1.49±0.13	1.34±0.15	1.34±0.07	0.124^{-}
2	2.15±0.18	1.73±0.16	2.10±0.14	1.92±0.16	1.97±0.08	0.246^{-}
3	2.69±0.20	2.31±0.18	2.52±0.15	2.55±0.20	2.52±0.09	0.536^{-}
4	3.30±0.20	3.20±0.22	3.27±0.18	3.21±0.28	3.24±0.11	0.988^{-}
5	4.42±0.22	4.32±0.24	4.01±0.19	4.26±0.38	4.25±0.13	0.741^{-}
6	6.14±0.34	6.17±0.31	5.31±0.30	5.84±0.61	5.87±0.20	0.422^{-}
EPEF						
1	210.26±3.46	215.01±3.91	213.20±4.73	214.88±2.93	213.34±1.88	0.799^{-}
2	264.73±7.60	266.00±7.09	252.44±6.61	256.61±8.34	259.95±3.71	0.518^{-}
3	315.99±4.81	335.64±6.21	322.37±8.22	334.01±7.79	327.00±3.50	0.142^{-}
4	354.81±6.19	358.24±4.29	334.00±13.07	362.79±3.49	352.46±3.99	0.051^{-}
5	350.33±6.26	362.01±7.04	358.98±4.72	372.07±5.45	360.85±3.04	0.085^{-}
6	350.98±5.88^{b}	355.09±8.26^{b}	353.21±6.27^{b}	383.38±8.56^{a}	360.67±3.89	0.007**

**:P<0.01, $^{-}$: Non significant, ^{a,b}:Means of groups followed by different letters differ significantly at p<0.05, **EPEF:** European Production Efficiency Factor

The effect of season on litter temperature was significant (P<0.05), with the highest litter temperatures observed in the summer flocks and the lowest in the spring and autumn flocks. Season significantly influenced the drinking water temperature on the first day (P<0.01), with the highest temperatures observed in the summer, autumn, and spring. Stocking density was not significantly affected by season (Table 4). Regardless of the season, the percentages of broilers with FPD severity scores of 0, 1, 2, 3, and 4 were 64.35, 15.65, 11.20, 5.75, and 3.05 %, respectively (Table 4). Summer flocks had the highest proportion (73.80 %) of broilers with healthy footpads (score 0). The highest ratios of broilers scored as scores 1 and 2 were observed in winter and Spring flocks, while the highest rate of broilers scored 3 was in the winter and autumn seasons. The most severe FPD (score 4) was observed in the winter and spring. As was the case for FPD, the prevalence of broilers with HB also varied from one season to the next. Overall, the proportions with HB severities 0, 1, 2, 3, and 4 were 62.60, 19.50, 14.95, 1.70 and 1.25% respectively. While the highest prevalence of broilers with HB 1 and 2 scores was observed in the winter and autumn, the highest rates of broilers with 3 scores were observed in the winter. The proportion of birds with an HB severity score of 4 was higher in winter and spring.

Table 4. The results on litter and drinking water temperatures, stocking density, and PFD and HB prevalence in seasons.

Traits	Score	Winter	Spring	Summer	Autumn	Total	P
Fist-day litter temperature (ºC)		31.93± 0.24^{ab}	31.59 ±0.13^{b}	32.44±0.14^{a}	31.77±0.21^{b}	31.93±0.10	0.013*
Fist-day water temperature (ºC)		25.05±0.38^{b}	25.88±0.20^{a}	26.25±0.13^{a}	25.94±0.23^{a}	25.78±0.13	0.009**
Stocking density (m^2/bird)		17.52±0.22	17.90±0.28	17.55±0.22	18.05±0.28	17.75±0.13	0.373^{-}
Stocking density (m^2/kg)		41.17±0.79	41.38±0.86	39.72±055	42.00±0.98	41.06±0.41	0.239^{-}
FPD (%)	0	55.60	62.40	73.80	65.60	64.35	
	1	19.00	16.00	12.80	14.80	15.65	
	2	14.40	11.40	9.00	10.00	11.20	
	3	7.20	5.80	3.40	6.60	5.75	
	4	3.80	4.40	1.00	3.00	3.05	
HB (%)	0	52.20	65.20	70.40	62.60	62.60	
	1	23.20	18.20	17.40	19.20	19.50	
	2	19.60	13.60	10.40	16.20	14.95	
	3	3.00	1.60	1.20	1.00	1.70	
	4	2.00	1.40	0.60	1.00	1.25	

*:P<0.05, **:P<0.01, $^{-}$: Non significant, ^{a,b}:Means of groups followed by different letters differ significantly at p<0.05, **FPD**: Food pad dermatitis; **HB**: hock burn.

Discussion and Conclusion

The season significantly influenced the ABW, TWG, TFI, and FCR. Moreover, summer flocks, in particular, exhibited the lowest TFI and TWG, likely due to the adverse impact of high ambient temperatures. The hot weather might have led the broilers to reduce TFI for thermoregulation purposes. The study found that litter and drinking water temperatures on the first day were notably higher in summer compared to other seasons. The lower TFI in summer could be attributed to increased ventilation and the cold air flow from the cooling pads used in the poultry house. The birds seemed to stay still and reluctant to move towards the feeders when the cool fresh air circulated inside the houses, possibly contributing to reduced TFI. These results indicated that the ventilation and cooling systems effectively maintained a suitable environment in the summer houses, ensuring good indoor air and litter quality. The research was carried out in poultry houses equipped with well-functioning ventilation systems and cooling pads, leading to the understanding that proper ventilation effectively prevents unfavourable conditions caused by high seasonal temperatures in the broiler house. This indoor air and litter quality maintenance likely contributed to the observation that disease score, FPD, and HB incidences were lowest in the summer flocks

(Musilová et al., 2013). Interestingly, despite slightly lower flock performance, the welfare of the broilers appeared to be better in summer, as indicated by the lower FPD and HB incidences. The low percentages of the broilers with FPD and HB indicated improved broiler welfare (De Jong et al., 2012; Youssef et al., 2021). These factors play a crucial role in determining the economic performance of the flocks (Kittelsen et al., 2017). Although no statistically significant difference was found between seasons, the fact that total mortality in summer flocks was slightly lower than in other seasons supports this interpretation (Table 2).

Winter flocks had high TFI; however, this did not result in a correspondingly high ABW. The winter conditions, characterized by high humidity and increased manure-origin gas emissions due to limited ventilation, might have created unfavorable indoor environments for the broilers, thus negatively impacting their health and welfare, ultimately leading to reduced flock performance (David et al., 2015). Shepherd and Fairchild (2010) and David et al. (2015) emphasized that low airflow rates during cold seasons contribute to elevated ammonia levels in the broiler house, leading to respiratory issues and decreased production performance.

The slightly higher mortality rate in winter flocks (6.14%) and the more frequent observation of respiratory and digestive diseases were thought to be responsible for this result. Moreover, the immune system responses of broilers raised in such conditions may have been compromised (Guo et al., 2023). Oakley et al. (2018) reported that the gastrointestinal microbiota of broilers varies with the season. Contrary to the findings of Petracci et al. (2006), although mortality was not significantly affected by the season, slightly higher mortality rates were observed in winter and spring flocks. The lower values for TWG and FCR in winter flocks may also be attributed to poor broiler welfare. De Jong et al. (2014) reported that housing broilers with poor litter quality decreased TWG and TFI, increased FCR, impaired bird performance, and increased hock burn, chest burns, and lameness resulting from higher litter moisture. Thus, our study observed the highest percentage of broilers with FPD and HB in winter flocks. Spring and autumn flocks exhibited higher TFI, TWG and EPEF compared to winter and summer flocks. This suggests that commercial broiler flocks achieve higher body weights by consuming more feed in these seasons, which do not experience extreme weather conditions (Youssef et al., 2021).

Moreover, the broiler ratios with FPD and HB were lower in spring and autumn flocks. This finding is expected since high ambient temperatures and adverse climatic conditions are less common in spring and autumn than in winter and summer (Aksoy et al., 2021). Surprisingly, however, the FCR was highest in the spring herds. This result contradicts the study's findings on spring mortality and animal welfare. It is challenging to explain this outcome, but it is possible that higher TFI (average 118 g) and mortality (0.33%), along with lower TWG (average 62.35 g) in spring flocks, may have influenced the FCR.

Consistent with previous research, the incidence of FPD and HB among broilers varied between seasons (De Jong et al., 2012; Shepherd and Fairchild, 2010). Winter flocks had the highest percentage of broilers with FPD and HB, possibly due to poor litter quality in the houses during that season. The litter quality was observed to deteriorate more rapidly in winter houses compared to other seasons, and certain areas, particularly in the latter part of the production period, had extremely compacted and hard litter. The study revealed that FPD and HB incidences were lower in the spring and autumn, which are a few months after or before winter, with the lowest occurrence of lesions observed during the summer. These findings may explain better foot and leg health during the spring and autumn, as litter quality tends to improve during these seasons, just like Meluzzi et al. (2008) reported significant positive correlations between litter moisture and FPD and HB incidences (r=0.87 and 0.75). While litter quality is essential, it is not the sole determinant of broiler foot health, as reported by reports like Kaukonen et al. (2016). There are many factors, such as litter type, ventilation, temperature, feed characteristics, water flowing from drinkers onto a litter, and intestinal disease, resulting in diarrhoea that can cause litter to become wet and compacted hard (De Jong et al., 2012; Musilová et al., 2013; Shepherd and Fairchild, 2010). FPD and HB in this study could be linked to respiratory and digestive system diseases in winter flocks, which are more vulnerable to diseases, leading to compromised foot and leg health due to reduced activity (De Jong et al., 2012). The percentages of birds with FPD and HB in this study (26.2-44.4% and 29.6-47.8%) were lower than those reported in other studies (Kaukonen et al., 2016; Musilová et al., 2013), possibly due to antibiotic treatment in winter flocks. The proportion of broilers with FPD and HB in winter, spring, and autumn flocks was higher than in summer flocks. Stocking density may also have played a role; stocking density in summer flocks was slightly lower than in other seasons (1.45-2.28 %). However, the prevalence of FPD and HB was lower in spring and autumn compared to winter, suggesting that foot pad and huck lesions are also influenced by environmental factors other than housing density (Jones et al., 2005; Meluzzi et al., 2008; Sans et al., 2021).

The study demonstrated that each season had varying effects on commercial broiler flocks' performance and welfare. The research findings conclude that the observed results can provide valuable guidance and contribute to developing innovative strategies for improving flock management and meeting animal welfare standards, considering different seasons' negative and positive effects.

Conflict of Interests

The authors have declared that they have no real, potential, or perceived conflicts of interest for this article.

Ethics Committee Approval

Ethics committee approval was received for this study from the ethics committee of Afyon Kocatepe University (Reference no: AKUHADYEK-88-21). Additionally, the authors declared that they comply with the Research and Publication Ethics.

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References

Aksoy T, Çürek Dİ, Narinç D, Önenç A, 2021: Effects of season, genotype, and rearing system on broiler chickens raised in different semi-intensive systems: performance, mortality, and slaughter results. *Trop Anim Health Prod*, 53(1), 1-11.

Aviagen, 2020: Ross Broiler Management Handbook.(htt https://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-Broiler-Pocket-Guide-2020-EN.pdf. Accessed 13 April 2022).

Beg MAH, Baqui MA, Sarker NR, Hossain MM, 2011: Effect of stocking density and feeding regime on performance of broiler chicken in summer season. *Int J Poult Sci*, 10(5),365-375.

David B, Mejdell C, Michel V, Lund V, Oppermann Moe R, 2015: Air quality in alternative housing systems may have an impact on laying hen welfare. Part II—Ammonia. *Animals*, 5(3), 886-896.

De Jong IC, Gunnink H, Van Harn J, 2014: Wet litter not only induces footpad dermatitis but also reduces overall welfare, technical performance, and carcass yield in broiler chickens. J *Appl Poult Res*, 23(1), 51-58.

De Jong IC, Van Harn J, Gunnink H, Lourens A, Van Riel JW, 2012: Measuring foot-pad lesions in commercial broiler houses. Some aspects of methodology. *Anim Welf*, 21(3), 325-330.

El-Tahawy AAS, Taha AE, Adel SA, 2017: Effect of flock size on the productive and economic efficiency of Ross 308 and Cobb 500 broilers. *Europ Poult Sci*, 81(176).

Federici JF, Vanderhasselt R, Sans ECO, Tuyttens FAM, Souza APO, Molento CFM, 2016: Assessment of broiler chicken welfare in Southern Brazil. *Braz J Poult Sci*, 18, 133-140.

Freeman N, Tuyttens FA, Johnson A, Marshall V, Garmyn A, Jacobs L, 2020: Remedying contact dermatitis in broiler chickens with novel flooring treatments. *Animals*, 10(10), 1761.

Guo Y, Zhang J, Li X, Wu J, Han J, Yang G, Zhang L, 2023: Oxidative stress mediated immunosuppression caused by ammonia gas via antioxidant/oxidant imbalance in broilers. *Br Poult Sci*, 64(1), 36-46.

Jones TA, Donnelly CA, Dawkins MS, 2005: Environmental and management factors affecting the welfare of chickens on commercial farms in the United Kingdom and Denmark stocked at five densities. *Poult Sci*, 84(8), 1155-1165.

Kaukonen E, Norring M, Valros A, 2016: Effect of litter quality on foot pad dermatitis, hock burns and breast blisters in broiler breeders during the production period. *Avian Pathology*, 45(6), 667-673.

Kittelsen KE, David B, Moe RO, Poulsen HD, Young JF, Granquist EG, 2017: Associations among gait score, production data, abattoir registrations, and postmortem tibia measurements in broiler chickens. *Poult Sci*, 96(5),1033-1040.

Kubiś M, Kołodziejski P, Pruszyńska E, Sassek M, Konieczka P, Górka P, FlagaJ Katarzynska-Banasik D, Hejdysz M, Szumacher M, Cie´slak, Kaczmarek SA, 2022: Combination of emulsifier and xylanase in wheat diets of broiler chickens. *Anim. Feed Sci. Technol*, 290, 115343.

Kryeziu AJ, Mestani N, Berisha S, Kamberi MA, 2018: The European performance indicators of broiler chickens as influenced by stocking density and sex. *Agron Res,* 16(2), 483 491.

Marcu A, Vacaru-Opriş I, Dumitrescu G, Ciochină LP, Marcu A, Nicula M, Nicula M, Pet I, Dronca D, Kelciov B, Mariş C, 2013: The influence of genetics on economic efficiency of broiler chickens growth. A*nim Sci Biotech*, 46(2),339-346.

Meluzzi A, Fabbri C, Folegatti E, Sirri F, 2008: Survey of chicken rearing conditions in Italy: effects of litter quality and stocking density on productivity, foot dermatitis and carcase injuries. *Br Poult Sci*, 49(3), 257-264.

Musilová A, Lichovníková M, Hampel D, Przywarová A, 2013: The effect of the season on incidence of footpad dermatitis and its effect on broilers performance. *Acta Univ Agric Silvic Mendelianae Brun*, 61, 1793-1798.

Nawaz AH, Amoah K, Leng QY, Zheng JH, Zhang WL, Zhang L, (2021). Poultry response to heat stress: Its physiological, metabolic, and genetic implications on meat production and quality including strategies to improve broiler production in a warming world. *Front Vet Sci*, 8, 699081.

Oakley BB, Vasconcelos EJ, Diniz PP, Calloway KN, Richardson E, Meinersmann RJ, Cox NA, Berrang ME, 2018: The cecal microbiome of commercial broiler chickens varies significantly by season. *Poult Sci*, 97(10), 3635-3644.

Petracci M, Bianchi M, Cavani C, Gaspari P, Lavazza A. 2006: Preslaughter mortality in broiler chickens, turkeys, and spent hens under commercial slaughtering. *Poult. Sci,* 85(9), 1660-1664.

Sans ECO, Tuyttens F, Taconeli CA, Rueda PM, Ciocca JR, Molento CFM, 2021: Welfare of broiler chickens reared under two different types of housing. *Anim Welf*, 30(3), 341-353.

Shepherd EM, Fairchild BD, 2010: Footpad dermatitis in poultry. *Poult Sci,* 89, 2043–2051.

Teyssier JR, Preynat A, Cozannet P, Briens M, Mauromoustakos A, Greene ES, Owens CM, Dridi S, Rochell, SJ. 2022: Constant and cyclic chronic heat stress models differentially influence growth performance, carcass traits and meat quality of broilers. *Poult Sci*, 101(8), 101963.

Ural A, Kılıç İ, 2013: Bilimsel Araştırma Süreci ve SPSS ile Veri Analizi. 4. Baskı, Detay Yayıncılık, Ankara.

Welfare Quality® 2009: Welfare Quality assessment protocol for poultry (broilers, laying hens). Welfare Quality® Consortium, Lelystad, Netherlands. https://edepot.wur.nl/233471. Accessed 07 March 2022.

Youssef SF, Abdelfattah MH, Bahnas MM, 2021: Effect of different seasons on growth performance, immune responses and antioxidant status of broiler chickens. *Egypt Poult Sci*, 41(1), 175-187.

HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Investigation of the Effects of Boric Acid Used in the Treatment of Rat Models with Knee Osteoarthritis Induced by Monosodium Iodoacetate on Liver Tissue

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Abstract: Osteoarthritis (OA) is a significant joint disease that can affect the health of individuals in many sectors. Although OA is primarily known as a joint disease in the field of health, it can also cause damage to organs such as the liver due to the systemic inflammatory reactions (SIRS) it triggers. Boric acid (BA), which has been the subject of many studies and has been shown to have anti-inflammatory, anti-apoptotic, antimicrobial, and anticancer properties, is widely used in various areas of the healthcare sector. In this study conducted for this purpose, the protective effect of BA against liver damage caused by OA was investigated. A total of 28 rats were divided into four groups, each consisting of 7 rats. Except for the control group, all animals were induced with monosodium iodoacetate (MIA) to develop OA, and then rats were administered doses of BA ranging 4 and 10 mg/kg for 21 days. In histopathological examinations, severe hepatitis and degenerative changes were observed in the liver tissue of the OA-induced group, while it was found that BA application reduced these damages dose-dependently. Similarly, in immunohistochemical analyses, it was observed that OA induction significantly increased the expression levels of TNF-α, IL-1β, iNOS, and MMP-13 in the liver, but in the groups treated with BA, this expression level significantly decreased depending on the dose. This study observed that BA exhibited anti-inflammatory properties and had a protective effect on liver tissue against SIRS caused by OA.

Keywords: *Boric acid, Histopathology, Immunohistochemistry, Monosodium iodoacetate, Osteoarthritis.*

Monosodyum İyodoasetat ile Oluşturulan Diz Osteoartriti Olan Sıçan Modellerinin Tedavisinde Kullanılan Borik Asitin Karaciğer Dokusu Üzerine Etkilerinin Araştırılması

Özet: Osteoartrit (OA), bireylerin sağlığını birçok sektörde etkileyebilen önemli bir eklem hastalığıdır. OA sağlık alanında öncelikle bir eklem hastalığı olarak bilinse de tetiklediği sistemik inflamatuar reaksiyonlar (SIRS) nedeniyle karaciğer gibi organlarda da hasara yol açabilmektedir. Birçok çalışmaya konu olan ve anti-enflamatuar, anti-apoptotik, anti-mikrobiyal ve anti-kanser özelliklere sahip olduğu gösterilen borik asit (BA), sağlık sektörünün çeşitli alanlarında yaygın olarak kullanılmaktadır. Bu amaçla yapılan bu çalışmada, BA'nın OA'nın neden olduğu karaciğer hasarına karşı koruyucu etkisi araştırılmıştır. Toplam 28 rat, her biri 7 rattan oluşan dört gruba ayrılmıştır. Kontrol grubu hariç, tüm hayvanlar monosodyum iyodoasetat (MIA) ile indüklenerek OA geliştirilmiş ve ardından ratlara 21 gün boyunca 4 ve 10 mg/kg dozlarda BA uygulanmıştır. Histopatolojik incelemelerde, OA oluşturulan grubun karaciğer dokusunda şiddetli hepatit ve dejeneratif değişiklikler gözlenirken, BA uygulamasının bu hasarları doza bağlı olarak azalttığı tespit edilmiştir. Benzer şekilde, immünohistokimyasal analizlerde, OA indüksiyonunun karaciğerde TNF-α, IL-1β, iNOS ve MMP-13 ekspresyon seviyelerini önemli ölçüde artırdığı, ancak BA ile tedavi edilen gruplarda bu ekspresyon seviyesinin doza bağlı olarak önemli ölçüde azaldığı gözlenmiştir. Bu çalışma, BA'nın anti-inflamatuar özellikler sergilediğini ve OA'nın neden olduğu SIRS'e karşı karaciğer dokusu üzerinde koruyucu bir etki gösterdiğini gözlemledi.

Anahtar Kelimeler: *Borik asit, Histopatoloji, İmmünohistokimya, Monosodyum iyodoasetat,Osteoartrit.*

Introduction

Osteoarthritis (OA) is a common disease among humans caused by damage to articular cartilage and the disruption of the balance in the production and degradation of extracellular matrix components, in which inflammatory substances that occur in response to this damage play an active role. OA is a joint disorder known for being both progressive and causing severe degeneration (Steels et al., 2019). With the formation of OA, subchondral bone thickening, synovial inflammation, osteophyte formation, thickening of the joint capsule and degenerative changes in the ligaments are seen in the knee joint. Depending on these symptoms, pain, stiffness and even disability may occur in the joints (Chen et al., 2017). Osteoarthritis is one of the most common musculoskeletal disorders worldwide, primarily affecting the knee region. The research conducted in 2020 reported that knee OA affects approximately 16% of the global population, with a higher prevalence in women compared to men, and its prevalence continues to rise due to modern lifestyle conditions and a high obesity rate (Cui et al., 2020). While OA is mostly recognized as a joint disease characterized by cartilage damage and loss, it has a complex pathogenesis that can affect all tissues in the body (Primorac et al., 2020). With the formation of osteoarthritis, the amount of proinflammatory cytokines in the environment is increased by the release of certain enzymes and inflammatory substances from the body. This picture shows inflammation and subsequent morphological differentiation of the joint tissue. In addition, some chemokines exert chemotactic effects on cells that secrete proinflammatory substances, attracting inflammatory cells to these regions and creating disadvantages in the treatment of osteoarthritis (Molnar et al., 2021).

Monosodium iodoacetate (MIA) is well-known for reproducing symptoms similar to those observed in patients with osteoarthritis (OA) when used to induce OA in animal models (Fonsi et al., 2019). When injected into the joint cavity, MIA disrupts glycolytic energy metabolism in chondrocytes, causing cell death, inflammation and cartilage damage. MIA induction in the OA model increases inflammatory cytokines and gradually leads to pain that results in nerve cell damage and neuralgia (Kuyinu et al., 2016).

Boron is an element found in Group 3A of the periodic table, possessing both semiconductor and metal characteristics. Due to its reactivity with oxygen, boron is found in nature as oxides of various elements rather than in its free form (Kılıçarslan, 2020). Boron is known to have a significant and wide-ranging role in the field of health, from cancer treatment to obesity (Başkan et al., 2022). Several studies have demonstrated that boric acid positively affects hormone levels such as steroids, thyroid hormones, estrogen, and testosterone, regulates various minerals including calcium and vitamin D, and helps maintain bone structure (Şaylı et al., 2000). It is also known to have a protective effect against atherosclerosis. Studies on humans have shown that boron compounds exhibit cytotoxic effects against cancer cells and reduce the risk of lung cancer, prostate cancer, and abnormal cervical cytology (Söğüt et al., 2020). Additionally, boron compounds are used in the treatment of conditions such as joint inflammation, osteoporosis, and depression, as they increase antioxidant enzyme activity and affect collagen enzymes to prevent premature aging (Acaroz et al., 2019; TMMOB, 2016).

In conclusion, although OA is primarily recognized as a joint disease, it is known to cause damage in many tissues and organs, including liver tissue, due to the systemic inflammatory reactions it induces. Boric acid is known as an anti-inflammatory agent widely used in the healthcare sector. This study aims to elucidate the inflammatory reactions occurring due to OA and the protective efficacy of boric acid against them.

Material and Methods

Animals and experimental model: Male albino Wistar rats, aged 12-16 weeks and weighing 250-300 grams, were obtained from the Animal Laboratory of Atatürk University. The rats were intraperitoneally administered ketamine (30 mg/kg) and 2% xylazine (6 mg/kg) to induce general anesthesia. Under anesthesia, 3 mg of monosodium iodacetate (MIA) dissolved in 0.9% NaCl (0.1 mL) solution was intraarticularly administered to the right patellar ligament of the rats as a single dose of 50 µL to create a knee osteoarthritis (OA) model.

The rats were randomly divided into four equal groups. Ethics Committee Approval: Atatürk University Animal Experiments Local Ethics Committee 01.04.2023 (Ethical Report:2023/04). Group 1 (Control; n=7x1): No procedure was performed on the rats in this group. Group 2 (Knee OA + Saline; n=7x1): After creating the knee OA model in the rats, 0.1 mL of saline was orally administered on days 1, 7, 14, and 21. Group 3 (Knee OA + 4 mg Boric Acid Oral; n=7x1): After creating the knee OA model in the rats, 1 mL of 4 mg/kg boric acid was administered orally once a day for three weeks. Group 4 (Knee OA + 10 mg Boric Acid Oral; n=7x1): After creating the knee OA model in the rats, 1 mL of 10 mg/kg boric acid was administered orally once a day for three weeks. After 24 hours of all treatments, the weights of the rats in each group were measured. They were then sacrificed under general anesthesia by decapitation following the intraperitoneal administration of ketamine hydrochloride (30 mg/kg) and 2% xylazine hydrochloride (6 mg/kg). Liver tissue samples were collected from the sacrificed rats to assess tissue damage. Liver tissues were placed in 10% formaldehyde solution.

Histopathological and immunohistochemical examination: Liver tissue samples obtained from the rats were routine tissue processing, the tissues were blocked with paraffin. The sections were stained with hematoxylin and eosin and subjected to histopathological evaluation under a light microscope. For immunohistochemical examination (IHC Detection Kit cat no: ab236466, abcam, UK), primary antibodies (TNF-α Cat No: sc-52746, Diluent Ratio: 1/100, US IL-1β Cat No: sc-52012, Diluent Ratio: 1/100,

US iNOS Cat No: sc-7271, Diluent Ratio: 1/100, US MMP-13 Cat No: sc-101564, Diluent Ratio: 1/100) were used. DAB (3,3'-Diaminobenzidine) chromogen was used to visualize the binding of primary and secondary antibodies. After the staining process, the necessary areas were captured using the Olympus BX51 with DP72 camera system. Based on histopathological findings and immunopositivity, the staining was scored as absent (-), mild (+, 5-10% cell), moderate (++, 15-20% cell), or intense (+++, >20% cell) (Iskender et al., 2022).

Statistical analysis: Statistical analysis of the histopathological evaluations was performed using the SPSS 13.0 program, and data were evaluated considering $p<0.05$ as statistically significant. Duncan's test was used for intergroup comparisons. The non-parametric Kruskal-Wallis test was used to determine group interactions, and the Mann-Whitney U test was used to identify differences between groups.

Results

Histopathological and immunohistochemical findings

Group 1: When liver tissues were histopathologically examined, it was determined that the tissues had a normal histological appearance (Figure 1a). When liver tissues were examined immunohistochemically, TNF-α, IL-1β, iNOS, and MMP-13 expressions were evaluated as negative in the tissues (Figure 2a, 3a, 4a, and 5a). **Group 2:** In the liver, severe hepatitis, severe degeneration and necrosis in hepatocytes, and moderate dilation in the sinusoidal space were observed (Figure 1b). When liver tissues were examined immunohistochemically, TNF-α and IL-1β expressions were observed in inflammatory cells, and iNOS and MMP-13 expressions were detected severely in hepatocytes (Figure 2b, 3b, 4b, and 5b). **Group 3:** In the liver, moderate hepatitis, severe degeneration in hepatocytes, and moderate dilation in the sinusoidal space were detected (Figure 1c). When liver tissues were examined immunohistochemically were detected that moderately TNF-α and IL-1β expressions were in inflammatory cells, and cytoplasmic iNOS and MMP-13 expressions moderately in hepatocytes (Figure 2c, 3c, 4c, and 5c). **Group 4:** In the liver, mild hepatitis, mild degeneration in hepatocytes, and mild dilation in the sinusoidal space were observed (Figure 1d). A significant difference ($p<0.05$) was found when Group 4 was compared with Group 2. The histopathological findings are summarized in Table 1. When liver tissues were examined immunohistochemically, TNF-α and IL-1β expressions were observed in inflammatory cells, while cytoplasmic iNOS and MMP-13 expressions were detected mildly in hepatocytes (Figure 2d, 3d, 4d, and 5d). A significant difference ($p<0.05$) was found when Group 4 was compared with Group 2. The immunohistochemical findings are summarized in Table 1.

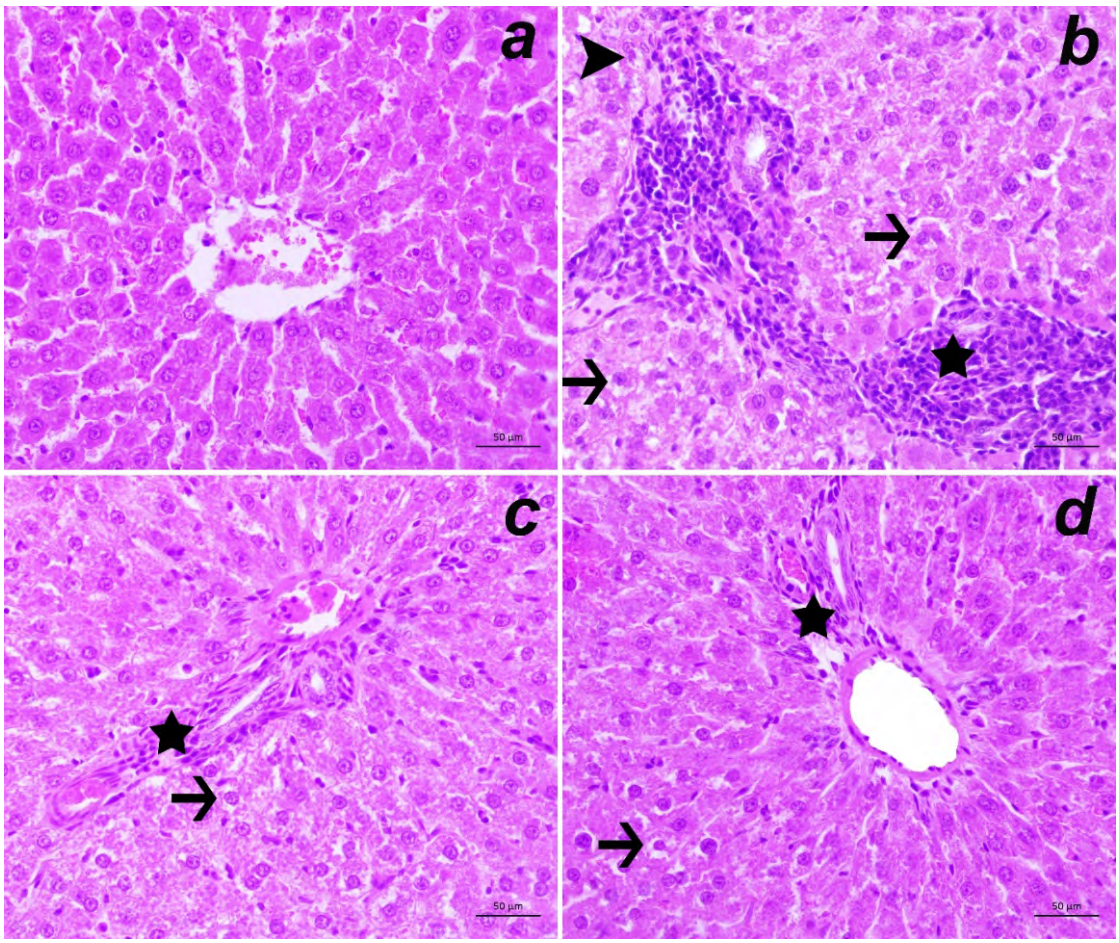

Figure 1. Liver tissue, Group 1(a), 2(b), 3(c), and 4(d), hepatitis (star), degeneration in hepatocytes (arrows), and necrosis (arrowhead), H&E, Scale bar: 50µm.

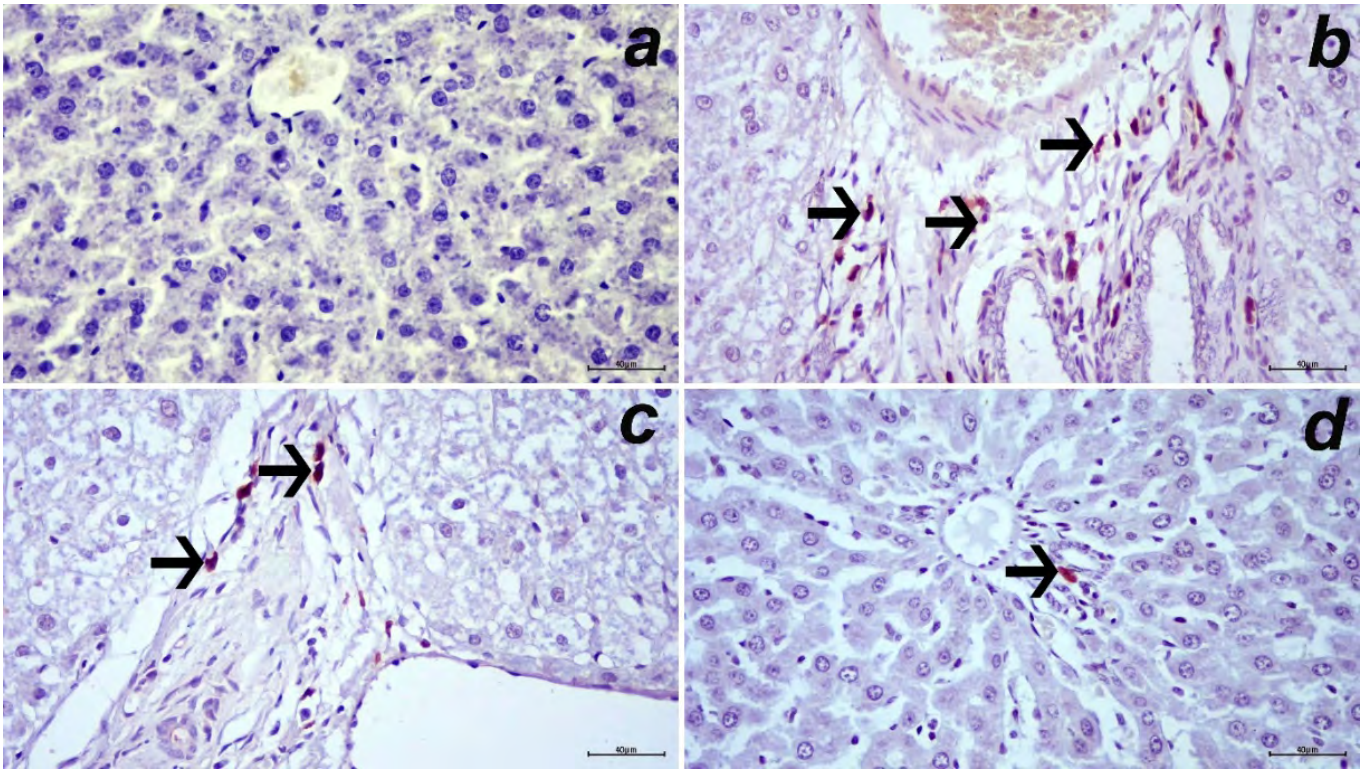

Figure 2. Liver tissue, Group 1(a), 2(b), 3(c), and 4(d), intracytoplasmic TNF-α expressions in inflammatory cells (arrows), IHC-P, Scale bar: 40µm.

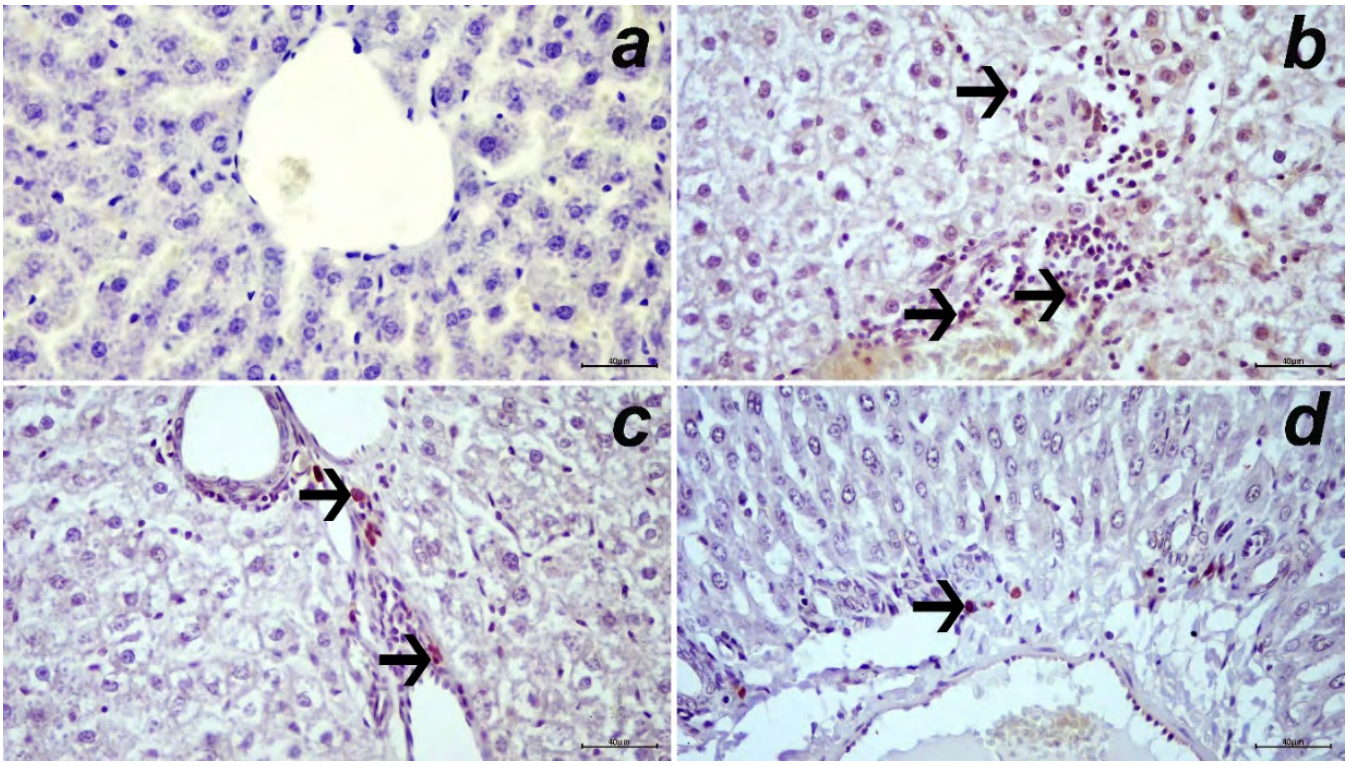

Figure 3. Liver tissue, Group 1(a), 2(b), 3(c), and 4(d), intracytoplasmic IL-1β expressions in inflammatory cells (arrows), IHC-P, Scale bar: 40µm.

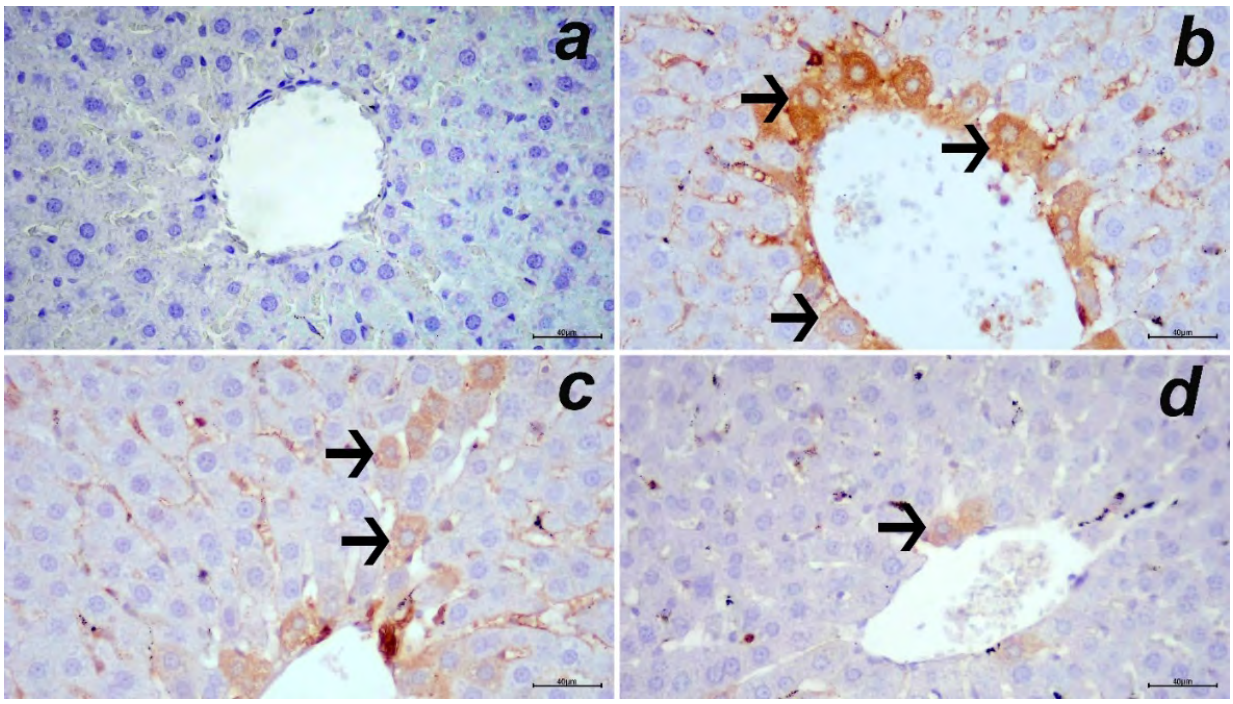

Figure 4. Liver tissue, Group 1(a), 2(b), 3(c), and 4(d), intracytoplasmic iNOS expressions in hepatocytes (arrows), IHC-P, Scale bar: 40µm.

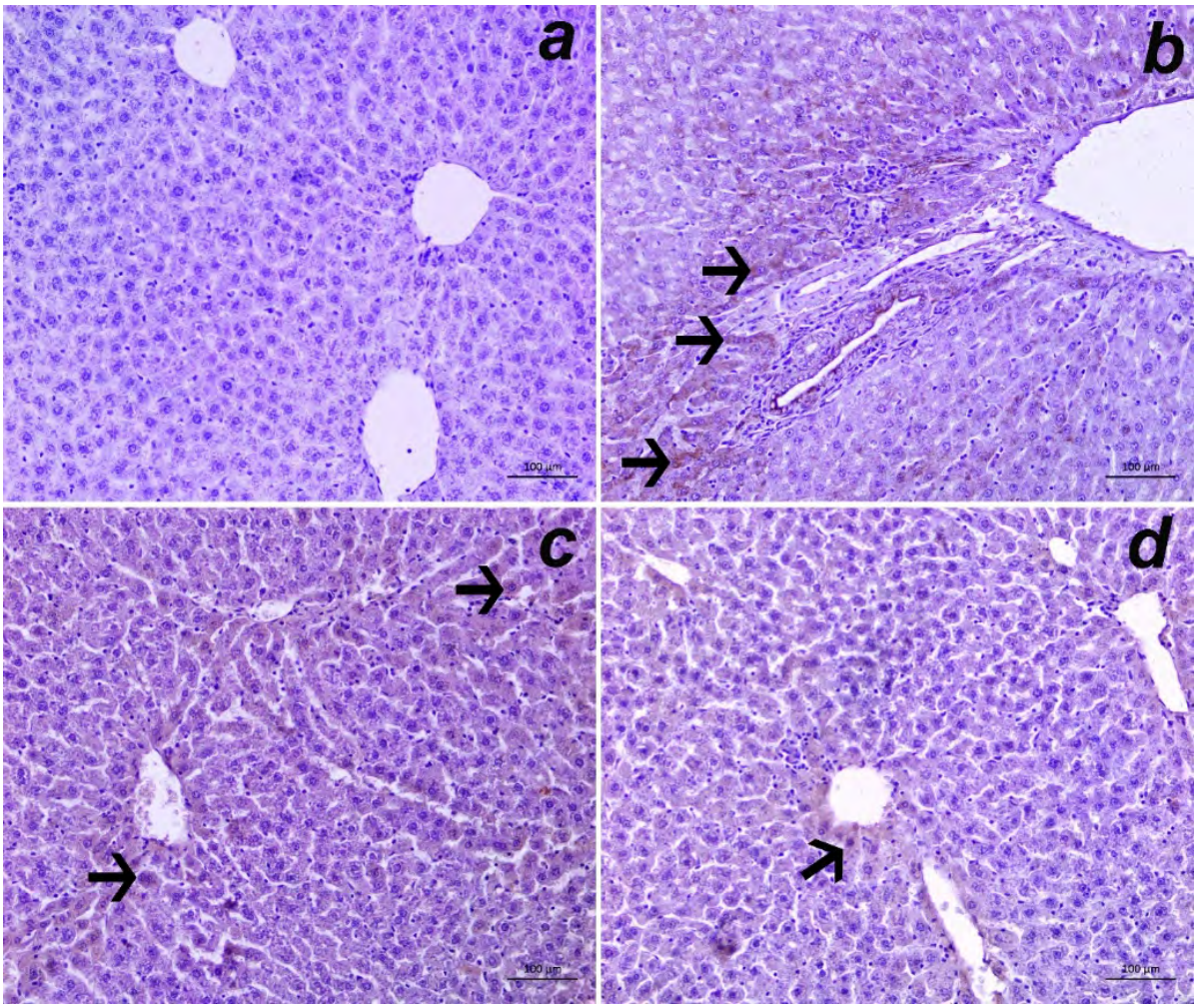

Figure 5. Liver tissue, Group 1(a), 2(b), 3(c), and 4(d), intracytoplasmic MMP-13 expressions in hepatocytes (arrows), IHC-P, Scale bar: 100µm.

Table 1. Histopathological and immunohistochemical findings in liver tissue and their scoring.

	Group 1	Group 2	Group 3	Group 4
Hepatitis	-	+++	++	+
Degeneration in hepatocytes	-	+++	+++	+
Necrosis in hepatocytes	-	+	-	-
Dilatation of sinusoids	-	++	++	+
TNF-α expressions	-	+++	++	+
IL-1β expressions	-	+++	++	+
iNOS expressions	-	+++	++	+
MMP-13 expressions	-	+++	++	+

Discussion and Conclusion

Boric acid is a mineral with antioxidant properties. It is widely used in various fields, including traditional healthcare, industry, and agriculture. Two hypotheses regarding the biochemical and physiological role of boric acid in living organisms have been proposed in the literature. Firstly, it acts as a response to hormone effects and cell membrane functions, affecting both transmembrane signaling and the movement of regulatory ions (Cengiz et al., 2018; Ince et al., 2012; Sogut et al., 2018). Secondly, boric acid can serve as a metabolic regulator in certain enzymatic systems. It increases the level of reduced glutathione, thereby reducing the effects of oxidative damage in the body and inhibiting the production of reactive oxygen species (ROS) and apoptosis (Ince et al., 2014). In a study conducted by Ince et al., it was found that boric acid exhibited strong hepatoprotective effects on liver damage. They demonstrated the protective efficacy of boric acid administration against histopathological changes induced by carbon tetrachloride in rat liver tissue. They suggested that this effect was due to both the activity of the antioxidant defense system and the inhibition of lipid peroxidation (Ince et al., 2012). Similarly, in a study by Cengiz et al., the protective effect of boric acid against damage induced by cyclophosphamide was emphasized (Cengiz et al., 2019). Based on these studies and literature findings, it is believed that boric acid has a protective effect against various types of liver damage. In accordance with the literature, the results of our study showed that boric acid showed antioxidant and antifilamatory activity in liver tissue against systemic inflammatory reactions in OA model.

Although there are few studies investigating the systemic inflammatory response in liver tissue associated with osteoarthritis (OA), no studies specifically examining boric acid in this context have been found. Some studies have indicated the occurrence of systemic inflammatory reactions in liver tissue associated with OA (Stammers et al., 1992). Additionally, studies on reperfusion-induced liver damage have shown that reperfusion, along with macrophage activation, leads to an increase in pro-inflammatory cytokine levels. The increased levels of pro-inflammatory cytokines such as IL-6 and TNF-α, which play a significant role in reperfusion injury, contribute to damage both in the reperfused tissue and in distant organs (Yildar et al., 2014). In an experimental study that induced hepatic ischemia-reperfusion injury, Crockett et al. observed histopathological findings such as sinusoidal congestion, cytoplasmic vacuolization, and neutrophil infiltration in the liver tissue (Crockett et al., 2006). It has been reported in the literature that inflammation occurs in liver tissues due to systemic inflammation. In this study, it was revealed that systemic inflammatory reactions occurring in the OA model also caused inflammatory reactions in the liver tissues of rats. Again, it was observed that proinflammatory cytokines IL-1β and TNF-α increased due to the increase in iNOS in liver tissues, and as a result, MMP-13 expression from hepatocytes also increased significantly. It is thought that this increase in MMP-13 develops due to systemic inflammatory response syndrome (SIRS) in the body.

No study investigating the effects of boric acid on liver damage in an OA model has been found. However, in a study conducted by Başbuğ et al., it was demonstrated that boric acid exhibited anti-inflammatory activity and protected liver tissue against inflammatory reactions induced by ischemia-reperfusion injury (Başbuğ et al., 2015). Similarly, in a study where ethanol-induced liver damage was examined, it was found that ethanol increased the sensitivity of Kupffer cells and the expression of inflammatory cytokines such as TNF-α (Dai et al., 2003). In a similar study on ethanol-induced liver injury, it was reported that boric acid application suppressed the increased expression of TNF-α (Sogut et al., 2018). These studies indicate that boric acid exhibits anti-inflammatory activity against inflammatory reactions occurring in the liver tissue associated with both ischemia-reperfusion injury and ethanol-induced liver damage. In this study, it was observed that TNF-α expressions due to inflammatory reactions in the liver tissue in the OA model were severely increased, and these expressions were significantly decreased due to boric acid administration. This is thought to be due to the anti-inflammatory activity of boric acid.

In conclusion, this study demonstrated that the systemic inflammatory response associated with the development of osteoarthritis induced by MIA leads to liver damage, and boric acid treatment exhibits a protective effect

against SIRS in the liver tissue. Furthermore, based on the obtained results, it is suggested that boric acid may have a protective effect against SIRS associated with OA in various tissues and organs.

Conflict of Interest

The authors stated that they did not have anyreal, potential or perceived conflict of interest.

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Author contribution

Idea, concept and design: I.B, K.A.T.K
Data collection and analysis: I.B, K.A.T.K, E.M.K
Drafting of the manuscript: I.B, G.G, K.G, S.Y.T
Critical review: I.B, K.A.T.K, F.D.M

References

Acaroz U, Kara R, Gurler Z, Ince S, Demirel HH, Acaroz DA Kucukkurt I, Eryavuz A, Varol N, Zhu K, 2019: Bisphenol-A induced oxidative stress, inflammatory gene expression, and metabolic and histopathological changes in male Wistar albino rats: protective role of boron. *Tox Res*, 8(2), 262-269.

Başbuğ M, Yıldar M, Yaman I, Özkan ÖF, Akşit H, Cavdar F, Sunay FB, Ozyıgıt MO, Derici H, 2015: Effects of boric acid in an experimental rat model of hepatic ischemia-reperfusion injury. *Acta Med Mediterr*, 31, 1067.

Başkan S, Kanak EK, Yılmaz SÖ, 2022: Borun Antimikrobiyel Etkileri ve Gıdalarda Koruyucu Olarak Kullanım Olanaklarının Değerlendirilmesi. *Gıda*, 47(3), 399-407.

Cengiz M, 2018: Boric acid protects against cyclophosphamide-induced oxidative stress and renal damage in rats. *CBM,* 64(12), 11-14.

Cengiz M, Yildiz SC, Demir C, Şahin İK, Teksoy Ö, Ayhanci A, 2019: Hepato-preventive and anti-apoptotic role of boric acid against liver injury induced by cyclophosphamide. *J Trace Elem Med Biol*, 53, 1-7.

Chen DI, Shen J, Zhao W, Wang T, Han L, Hamilton JL, Im H, 2017: Osteoarthritis: toward a comprehensive understanding of pathological mechanism. *Bone Res*, *5*(1), 1-13.

Crockett ET, Galligan JJ, Uhal BD, Harkema J, Roth R, Pandya K, 2006: Protection of early phase hepatic ischemia-reperfusion injury by cholinergic agonists. *BMC Clinic Patho*, 6(1), 1-13.

Cui A, Li H, Wang D, Zhong J, Chen Y, Lu H, 2020: Global, regional prevalence, incidence and risk factors of knee osteoarthritis in population-based studies. *eClinicalMedicine*, 29, 100587.

Dai LL, Gong JP, Zuo GQ, Wu CX, Shi YJ, Li XH, Peng Y, Deng W, Li SW, Liu C.A, 2003: Synthesis of endotoxin receptor CD14 protein in Kupffer cells and its role in alcohol-induced liver disease. *WJG*, 9(3), 622.

Fonsi M, El Amrani AI, Gervais F, Vincent P, 2020: Intra-articular hyaluronic acid and chondroitin sulfate: pharmacokinetic investigation in osteoarthritic rat models. *CTR*, 92, 100573.

Ince S, Keles H, Erdogan M, Hazman O, Kucukkurt I, 2012: Protective effect of boric acid against carbon tetrachloride–induced hepatotoxicity in mice. *Drug Chem Toxicol*, 35(3), 285-292.

Ince S, Kucukkurt I, Demirel HH, Acaroz DA, Akbel E, Cigerci IH, 2014: Protective effects of boron on cyclophosphamide induced lipid peroxidation and genotoxicity in rats. *Chemosphere*, 108, 197-204.

Iskender H, Dokumacioglu E, Terim Kapakin KA, Yenice G, Mohtare B, Bolat I, Hayirli A, 2022: Effects of oleanolic acid on inflammation and metabolism in diabetic rats. *Bio and Histo,* 97(4), 269-276.

Kılıçaslan C, 2020: Borun Stratejik ve Ekonomik Önemi. Doktora tezi, Costa Rica Universidad Empresarial De Costa Rica (Unem), İstanbul.

Kuyinu EL, Narayanan G, Nair LS, Laurencin CT, 2016: Animal models of osteoarthritis: classification, update, and measurement of outcomes. *J Orthop Surg Res*, 11(1), 1-27.

Molnar V, Matisic V, Kodvanj I, Bjelica R, Jelec Z, Hudetz D, Rod E, Cukelj F, Vrdoljak T, Vidovic D, Staresinic M, Sabalic S, Dobricic B, Petrovic T, Anticevic D, Boric I, Kosir R, Primorac D, 2021: Cytokines and chemokines involved in osteoarthritis pathogenesis. *Int J Mol Sci*, 22(17), 9208.

Primorac D, Molnar V, Rod E, Jelec Z, Cukelj F, Matisic V, Vrdoljak T, Hudetz D, Hajsok H, Boric I, 2020: Knee Osteoarthritis: A Review of Pathogenesis and State-Of-The-Art Non-Operative Therapeutic Considerations. *Genes*, 11(8), 854.

Sogut I, Paltun SO, Tuncdemir M, Ersoz M, Hurdag C, 2018: The antioxidant and antiapoptotic effect of boric acid on hepatoxicity in chronic alcohol-fed rats. *J Physiol Pharmacol*, 96(4), 404-411.

Söğüt Ö, Acar O, 2020: Bor ve sağlık. *Lit Ecz Bil Derg*, 9(1), 11-7.

Stammers T, Sibbald B, Freeling P, 1992: Efficacy of cod liver oil as an adjunct to non-steroidal anti-inflammatory drug treatment in the management of osteoarthritis in general practice. *ARD*, 51(1), 128-129.

Steels E, Venkatesh R, Steels E, Vitetta G, Vitetta L, 2019: A double-blind randomized placebo-controlled study assessing safety, tolerability and efficacy of palmitoylethanolamide for symptoms of knee osteoarthritis. *Inflammopharmacology*, 27, 475-485.

Şaylı BS, 2000: İnsan Sağlığı ve Bor Mineralleri. AÜ Tıp Fakültesi-Eti Holding Araştırma Projeleri. Ankara. Mayıs.

TMMOB 2016. Bor Raporu, ISBN 978-605-01- 0883-5. 2016. Ankara.

Yildar M, Aksit H, Korkut O, Ozyigit MO, Sunay B, Seyrek K, 2014: Protective effect of 2-aminoethyl diphenylborinate on acute ischemia–reperfusion injury in the rat kidney. *J Surg Res,* 187(2), 683-689.

HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Marmara Bölgesi'ndeki Küçükbaş ve Büyükbaş Hayvan Varlığının Basit Uyum Analizi ile İncelenmesi

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Özet: Bu çalışmanın amacı basit uyum analizini kullanarak Marmara Bölgesi'nde bulunan küçükbaş ve büyükbaş hayvan dağılımını illere göre detaylı bir şekilde analiz etmektir. Basit uyum analizi, kategorik olarak elde edilmiş ya da elde edildikten sonra kategorize edilerek iki boyutlu çapraz tablo haline getirilmiş verilerin analizinde kullanılan bir yöntemdir. Çalışmanın araştırma materyalini TÜİK'in veri tabanından alınan hayvancılık verileri oluşturmaktadır. Küçükbaş hayvanlar için Merinos koyunu, yerli ve diğer ırk koyunlar ile keçi sayıları, büyükbaş hayvanlar için ise yerli sığır, kültür sığır ve manda sayıları kullanılmıştır. Uyum analizi sonuçlarına göre küçükbaş hayvanlar için toplam değişkenliğin %11,7'si, büyükbaş hayvanlar için ise %7,1'i ilk iki boyut tarafından açıklanmıştır. Buna göre; Bursa, Balıkesir ve Tekirdağ'ın Merinos koyunu yetiştiriciliği, Kocaeli, Kırklareli ve Sakarya'nın yerli ve diğer ırk koyun yetiştiriciliği ve Çanakkale'nin ise keçi yetiştiriciliğinde Marmara bölgesindeki küçükbaş hayvancılık sektörüne önemli katkı sağladığı söylenebilir. Büyükbaş hayvanlar için Bursa, Balıkesir ve Sakarya'nın kültür sığırı yetiştiriciliği konusunda bölgeye önemli katkı sağladığı görülmektedir. İstatistiksel değerlendirmenin yanında grafiksel olarak yorum sağladığı için uyum analizi yönteminin hayvancılık ile ilgili yapılan çalışmalarda kullanılmasının faydalı olacağı düşünülmektedir.

Anahtar Kelimeler: *Kategorik Değişken, Keçi, Koyun, Sığır, Uyum Analizi.*

Investigation of Small Ruminant and Cattle Livestock in the Marmara Region by Simple Correspondence Analysis

Abstract: This study aims to analyze the distribution of small ruminants and cattle in the Marmara Region in detail by provinces by using simple correspondence analysis. Simple correspondence analysis is a method used to analyze data obtained categorically or categorized after receiving and made into a two-dimensional cross-tabulation. The research material of the study consists of animal husbandry data obtained from the TurkStat database. The numbers of Merino sheep, domestic and other breeds of sheep and goats were used for small ruminants, and the numbers of domestic cattle, cultured cattle, and buffalo were used for cattle. According to the correspondence analysis results, the first two dimensions explained 11.7% of the total variability for small ruminants and 7.1% for cattle. According to this, it can be said that Bursa, Balıkesir, and Tekirdağ contributed remarkably to the sheep breeding sector in the Marmara region, in the breeding of Merino sheep, in the breeding of domestic and other breeds in Kocaeli, Kırklareli, and Sakarya, and in goat breeding in Çanakkale. For cattle, it is seen that Bursa, Balıkesir, and Sakarya make a significant contribution to the region in terms of culture cattle breeding. Since it provides graphical interpretation and statistical evaluation, it is thought that it will be helpful to use the correspondence analysis method in studies on animal husbandry.

Keywords: *Categorical variable, Cattle, Correspondence Analysis, Goat, Sheep.*

Giriş

Sürekli artan nüfusun sağlıklı ve yeterli beslenmesi için hayvancılığın önemi büyüktür. Hayvancılık sektörü toplumun et, süt ve yumurta gibi ihtiyaçlarını karşılayarak dengeli beslenmesinde önemli bir rol oynamaktadır. Bunun yanında yem, hayvansal ilaçlar ve hayvancılık ekipmanları gibi pek çok alanda istihdam alanı oluşturup ülke ekonomisine katkı sağlamaktadır (Ergün ve Bayram, 2021). Türkiye'nin coğrafi konumu, iklim koşulları ve doğal kaynakları ülkenin hayvancılık ve tarımsal üretiminde önemli bir yere sahiptir. 2021 yılı Türkiye İstatistik Kurumu (TÜİK) verilerine göre ülkede 18 milyon 36 bin adet büyükbaş ve 57 milyon 519 bin adet küçükbaş hayvan varlığına ulaşılmıştır. Türkiye'nin coğrafi yapısındaki farklılık, bölgeler arasında farklı hayvan türlerinin yetiştirilmesine neden olmaktadır (Demir, 2020; Polat, 2017).

Marmara Bölgesi, Türkiye'nin en gelişmiş ve yoğun nüfusa sahip bölgelerinden biridir (TÜİK, Nüfus kayıt sistemi, 2022). Bu bölgede hayvancılık sektörü de önemli bir yer tutar. Bölgede sığır, koyun, keçi ve manda yetiştiriciliği diğer bölgelere nazaran gerek hayvan verimi gerekse hayvan sayısı bakımından yüksek düzeydedir (Vural ve Fidan, 2007). Küçük aile işletmelerinin dışında yığınsal üretim yapan büyük işletmeler de bu bölgede fazladır.

Hayvancılık alanında yapılan araştırmaların sayısının arttırılmasının yanında bu alanda elde edilen verilerin kapsamlı bir şekilde analiz edilmesi de önemlidir (Demir, 2020). Diğer pek çok alanda olduğu gibi hayvanlar üzerinde yapılan çalışmalarda da kategorik verilerin analizine ihtiyaç duyulmaktadır. Kategorik veriler, kategori halinde gruplandırılabilen verilerdir. Kategorik yapıdaki verilerin analizinde kullanılan yöntemlerden biri de uyum analizidir. Uyum analizi, kategorik olarak sınıflandırılan verilerin yorumlanmasını sağlayan, çapraz tablolardaki değişkenler arasındaki farklılık ya da benzerlik ve ilişkileri açıklayan, kategorilerin birbirleri ile olan değişimlerini grafiksel olarak göstermeye olanak sağlayan çok değişkenli bir analiz yöntemidir (Özdamar, 2010; Suner ve Çelikoğlu, 2008). Uyum analizi, pek çok alanda kategorik verileri analiz etmek için tercih edilen bir yöntemdir (Aktürk, 2004; Gün ve Çavuş Erdem, 2014; Yılmaz ve Saraçlı, 2007). Hayvancılık alanında yapılan çalışmalarda genellikle frekans tabloları verilip bu tablolardaki sayı ve yüzde değerler üzerinden yorum yapılmaktadır (Demir, 2020). Ki-kare analizi ya da loglineer analiz yöntemleri ile analiz edildiğinde ayrıntılı bilgi elde edilemeyen kategorik verilerin çözümünde kullanılan uyum analizi yöntemi verilerin dağılımı ile ilgili herhangi bir varsayım gerektirmediği için tercih edilebilir. Uyum analizi yöntemi basit ve çoklu uyum analizi olmak üzere iki şekilde uygulanabilir (Alpar, 2011).

Hayvancılık alanında elde edilen kategorik verilerin uyum analizi ile değerlendirildiği çalışmaların az sayıda olması nedeniyle bu araştırmanın yapılmasına ihtiyaç duyulmuştur. Bu çalışmanın amacı, 2022 yılı için TÜİK veri tabanından elde edilen veriler yardımıyla Marmara Bölgesi'nde yer alan illerdeki büyükbaş ve küçükbaş hayvan varlığının basit uyum analizi yöntemi ile değerlendirilmesi ve grafiksel olarak incelenmesidir.

Materyal ve Metot

Bu çalışmada, araştırma materyali olarak Marmara Bölgesi'nde bulunan küçükbaş ve büyükbaş hayvan türlerinin illere göre sayıları kullanılmıştır. İlgili veriler Türkiye İstatistik Kurumu'nun açık erişimli resmi internet sitesinden alınmıştır (https://biruni.tuik.gov.tr/medas/?kn=101&locale=tr). Bu çalışma "Hayvan Deneyleri Etik Kurullarının Çalışma Usul ve Esaslarına Dair Yönetmelik" Madde 8 (k) gereği HADYEK iznine tabi değildir. Çalışmanın güncel olması amacıyla 2022 yılına ait veriler kullanılmıştır. Küçükbaş hayvanlar için yapılan değerlendirmede kategoriler Merinos koyunu, yerli ve diğer ırk koyunlar ve keçiler olarak belirlenmiştir. Büyükbaş hayvanlar için yerli sığır ırkı, kültür ırkları ve manda kategorileri için değerlendirme yapılmıştır. Hem küçükbaş hem de büyükbaş hayvanlarda üçer kategorinin olması TÜİK verilerindeki sınıflandırmadan kaynaklanmış ve ülke hayvancılığının genel özeti olarak düşünülmüştür.

Bu çalışmadaki verilerin değerlendirilmesinde basit uyum analizi kullanılmıştır. Bu yöntem, iki boyutlu kontenjans tablolarının ağırlıklı ana bileşenler analizini yapar. Uyum analizinde varyans kavramının yerine toplam inertia değerinin parçalanması amaçlanmaktadır (Alpar, 2011; Özdamar, 2010). Uyum analizinde Pearson ki-kare uzaklığı kullanılıyorsa bu ölçüyü doğrudan kullanmak yerine tablonun toplam ki-kare değerine oranlanmış biçimi olan inertia değeri kullanılır ($\text{İ}nertia = \chi_{ij}^2 / \sum\sum \chi_{ij}^2$). Eşitliğin payı i. satır ve j. sütundaki gözleme ait ki-kare değerini, paydası ise toplam ki-kare değerini göstermektedir. Inertia değeri, her bir gözenin oranlanmış ki-kare değeridir ve her bir hücre ya da sıranın ki-kare değerlerine bağlı olarak değişken kategorilerinin birbirleri ile olan ilişkisinin ölçüsüdür (Özdamar, 2010).

Çapraz tablo olarak gösterilen (r*c boyutlu) X ve Y değişkenlerinin basit uyum analizini yapmak için öncelikle tablonun her bir hücresinde yer alan frekansların marjinal gözlenme frekansları hesaplanır. Daha sonra tablonun her bir satır ve sütunundaki marjinal olasılıklar hesaplanır. Elde edilen sıra marjinal olasılıklarına sıra profilleri, sütun toplamlarına göre elde edilen profillere ise sütun profilleri adı verilir. Her bir sıra ve sütun toplamlarının genel toplama bölünmesi ile elde edilen oranlara ise mass (kategori olasılığı) adı verilir (Alpar, 2011; Özdamar, 2010). Her bir kategorinin birbirlerinden uzaklıkları ki-kare uzaklığı ya da Öklid uzaklığı olarak hesaplanabilir (Özdamar, 2010). Satır ve sütun profillerinin hesaplanmasından sonra satır ve sütun değişkenleri için elde edilen tüm noktalara en yakın olan min[(satır sayısı-1), (sütun sayısı-1)] boyutlu indirgenmiş uzaydaki koordinatlar bulunur ve noktalar grafik üzerinde gösterilir (Alpar, 2011).

Bu çalışmada hem küçükbaş hayvanların hem de büyükbaş hayvanların illere göre dağılımı ayrı ayrı incelenmiş ve verilerin analizinde IBM SPSS Statistics 21 programı kullanılmıştır.

Bulgular

Uyum analizinin ilk aşamasında hesaplanan satır ve sütun profillerine ilişkin sonuçlar küçükbaş hayvanlar için Tablo 1'de, büyükbaş hayvanlar için Tablo 2'de verilmiştir. Tablo 1'de yer alan satır profillerine göre Marmara Bölgesinde küçükbaş hayvancılık sektörünün %22'sini Merinos koyunu, %61,5'ini yerli ve diğer ırk koyunlar ve %16,4'ünü ise keçi oluşturmaktadır. Yalova ili, yerli ve diğer ırk koyun varlığı açısından %88,3 ile en yüksek potansiyele sahipken; Merinos koyunu açısından %0,04 ile çok düşük bir potansiyel oluşturmaktadır. Tablo 1'de yer alan sütun profillerine göre küçükbaş hayvan yetiştiriciliğinde en yüksek payı oluşturan ilk üç il sırasıyla Balıkesir (%30,8), Çanakkale (%17,7) ve Bursa (%12,8)'dır.

Tablo 1. Marmara Bölgesi'nde bulunan illerdeki küçükbaş hayvan grupları için elde edilen satır ve sütun profilleri.

	Satır Profilleri				Sütun Profilleri			
İller	**Merinos**	**Yerli**	**Keçi**	**Toplam**	**Merinos**	**Yerli**	**Keçi**	**Ortalama Sütun Profili**
Balıkesir	0,321	0,566	0,113	1,000	0,450	0,284	0,212	0,308
Bilecik	0,084	0,695	0,221	1,000	0,016	0,046	0,055	0,041
Bursa	0,371	0,500	0,128	1,000	0,216	0,104	0,100	0,128
Edirne	0,257	0,595	0,148	1,000	0,105	0,087	0,081	0,090
Kocaeli	0,117	0,702	0,181	1,000	0,014	0,031	0,030	0,027
Kırklareli	0,102	0,744	0,154	1,000	0,040	0,104	0,081	0,086
Sakarya	0,052	0,756	0,192	1,000	0,005	0,026	0,025	0,021
Tekirdağ	0,321	0,555	0,124	1,000	0,110	0,068	0,057	0,075
Yalova	0,004	0,883	0,113	1,000	0,000	0,009	0,004	0,006
Çanakkale	0,047	0,652	0,301	1,000	0,038	0,188	0,325	0,177
İstanbul	0,033	0,840	0,128	1,000	0,006	0,052	0,030	0,038
Ortalama Satır Profili	0,220	0,615	0,164	-				
Toplam					1,000	1,000	1,000	-

Tablo 2. Marmara Bölgesi'nde bulunan illerdeki büyükbaş hayvan grupları için elde edilen satır ve sütun profilleri.

	Satır Profilleri				Sütun Profilleri			
İller	**Yerli (Sığır)**	**Kültür (Sığır)**	**Manda**	**Toplam**	**Yerli (Sığır)**	**Kültür (Sığır)**	**Manda**	**Ortalama Sütun Profili**
Balıkesir	0,067	0,922	0,011	1,000	0,407	0,272	0,164	0,276
Bilecik	0,020	0,979	0,001	1,000	0,008	0,020	0,001	0,019
Bursa	0,046	0,935	0,019	1,000	0,130	0,129	0,139	0,129
Edirne	0,005	0,992	0,003	1,000	0,009	0,091	0,014	0,086
Kocaeli	0,070	0,897	0,033	1,000	0,087	0,054	0,105	0,057
Kırklareli	0,001	0,986	0,013	1,000	0,002	0,089	0,060	0,084
Sakarya	0,057	0,932	0,010	1,000	0,120	0,095	0,055	0,096
Tekirdağ	0,007	0,981	0,012	1,000	0,013	0,085	0,054	0,081
Yalova	0,091	0,907	0,002	1,000	0,013	0,006	0,001	0,006
Çanakkale	0,074	0,926	0,000	1,000	0,191	0,116	0,002	0,118
İstanbul	0,018	0,832	0,150	1,000	0,020	0,043	0,406	0,049
Ortalama Satır Profili	0,046	0,936	0,018	-				
Toplam					1,000	1,000	1,000	-

Tablo 2'de yer alan satır profillerine göre bölgede büyükbaş hayvancılık sektörünün %4,6'sını yerli sığır ırkı, %93,6'sını kültür ırkı ve %1,8'ini ise manda oluşturmaktadır. Edirne'de kültür ırkları %99,2 ile en yüksek ve İstanbul'da ise %83,2 ile en düşük orana sahiptir. Tablo 2'de yer alan sütun profillerine göre büyükbaş hayvan yetiştiriciliğinde en yüksek payı oluşturan ilk üç il sırasıyla Balıkesir (%27,6), Bursa (%12,9) ve Çanakkale (%11,8) 'dir.

Marmara Bölgesi'nde bulunan illere göre küçükbaş ve büyükbaş hayvan sayılarının farklı olup olmadığını belirlemek için yapılan uyum analizi sonucunda elde edilen boyutlar ve inertia değerleri sırasıyla Tablo 3 ve Tablo 4'te yer almaktadır. Küçükbaş hayvanlar için elde edilen analiz sonuçlarına göre, 1. ve 2. boyuta ait tekil değerler sırası ile 0,322 ve 0,116 olarak bulunmuştur. Inertia değerleri ise sırası ile 0,103 ve 0,013 olarak elde edilmiştir. Uyum analizinde varyans yerine kullanılan inertia kavramı toplam değişkenliğin %10,3'ünün birinci boyut, %1,3'ünün ise ikinci boyut tarafından açıklandığını göstermektedir. Yani ilk iki boyutun birlikte varyansı açıklama oranı %11,7'dir. Bunun yanında birinci boyutun inertiadaki payı %88,5 iken ikinci boyutun payı %11,5'tir. Ki-kare test istatistiğine ait olasılık değerinin $p<0,001$ olarak elde edilmesi küçükbaş hayvan yetiştiriciliğinin illere göre farklılık gösterdiği anlamına gelmektedir.

Büyükbaş hayvanlar için elde edilen analiz sonuçlarına göre, 1. ve 2. boyuta ait tekil değerler sırası ile 0,233 ve 0,129 olarak bulunmuştur. Inertia değerleri ise sırası ile 0,054 ve 0,017 olarak elde edilmiştir. Uyum analizinde varyans yerine kullanılan inertia kavramı toplam değişkenliğin %5,4'ünün

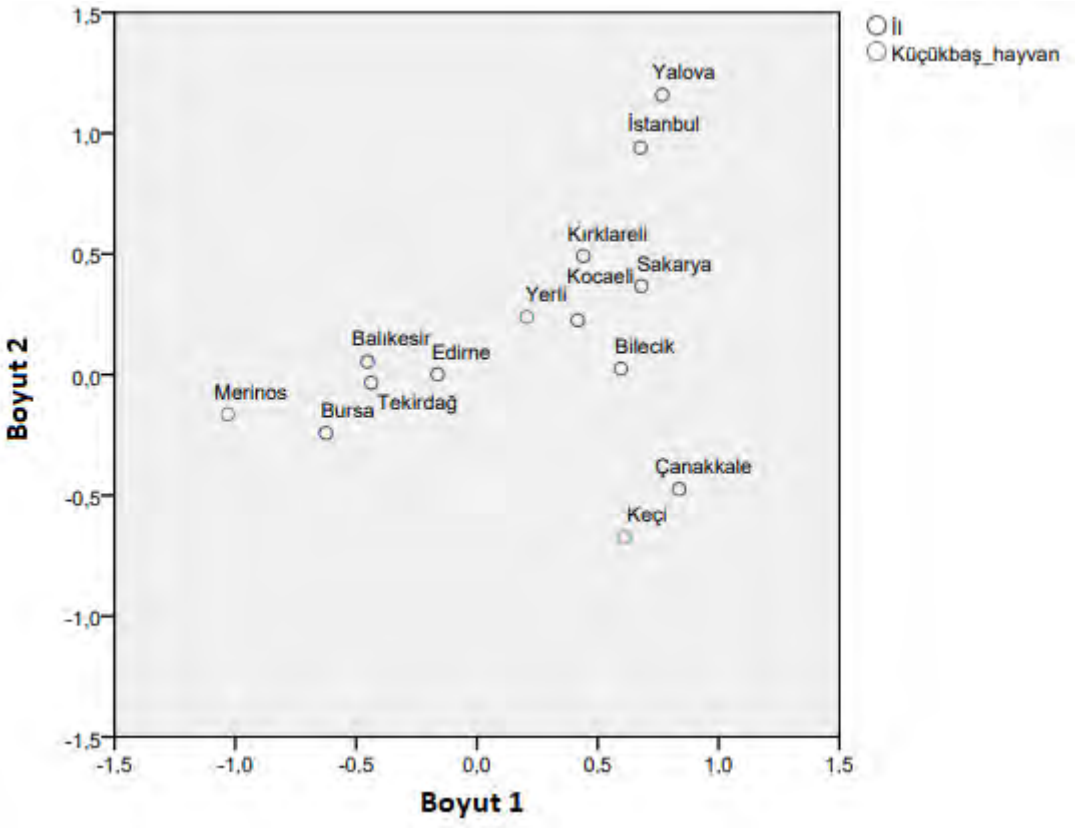
Tablo 3. Boyutların özet tablosu ve açıklanan inertia değerleri (küçükbaş hayvan).

					Açıklanan Inertia	
Boyut	Tekil Değer	Inertia	Ki-kare	p	Açıklanan (%)	Birikimli (%)
1	0,322	0,103			0,885	0,885
2	0,116	0,013			0,115	1,000
Toplam		0,117	543569,951	<0,001	1,000	1,000

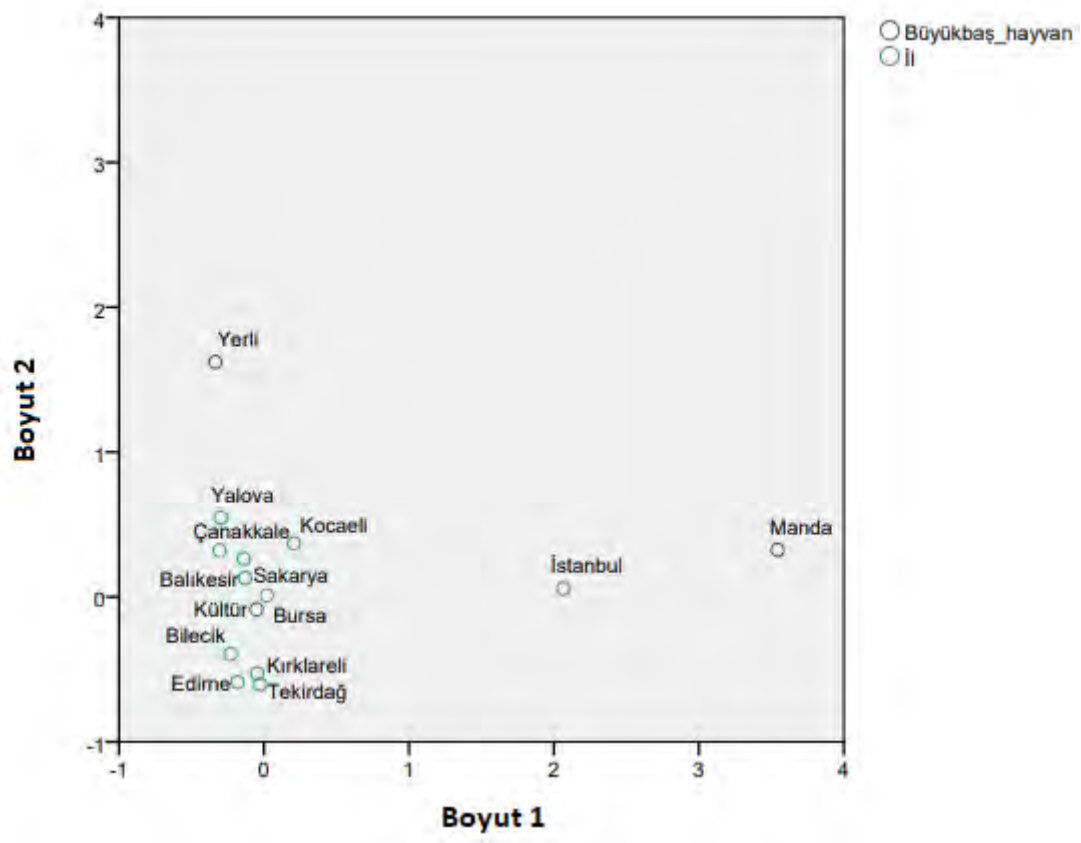
Tablo 4. Boyutların özet tablosu ve açıklanan inertia değerleri (büyükbaş hayvan).

					Açıklanan Inertia	
Boyut	Tekil Değer	Inertia	Ki-kare	p	Açıklanan (%)	Birikimli (%)
1	0,233	0,054			0,766	0,766
2	0,129	0,017			0,234	1,000
Toplam		0,071	126271,856	<0,001	1,000	1,000

birinci boyut, %1,7'sinin ise ikinci boyut tarafından açıklandığını göstermektedir. Yani ilk iki boyutun birlikte varyansı açıklama oranı %7,1'dir. Bunun yanında birinci boyutun inertiadaki payı %76,6 iken ikinci boyutun payı %23,4'tür. Ki-kare test istatistiğine ait olasılık değerinin p<0,001 olarak elde edilmesi büyükbaş hayvan yetiştiriciliğinin illere göre farklılık gösterdiği anlamına gelmektedir. Değişkenlerin konumu ve iki değişkenin kategorileri arasındaki ilişki küçükbaş hayvanlar için Şekil 1'de, büyükbaş hayvanlar için Şekil 2'de verilmiştir.



Şekil 1. Marmara Bölgesi'nde bulunan illerdeki küçükbaş hayvan grupları için uyum analizi diyagramı.



Şekil 2. Marmara Bölgesi'nde bulunan illerdeki büyükbaş hayvan grupları için uyum analizi diyagramı.

Tartışma ve Sonuç

Bu çalışmada, Marmara bölgesinde bulunan illere göre küçükbaş ve büyükbaş hayvanların dağılımı basit uyum analizi ile incelenmiştir. Uyum analizi yöntemi, kategorik olarak elde edilmiş veriler için ki-kare analizinin hücrelerdeki frekansların yetersizliği nedeniyle kullanılamadığı ya da değişken kategorileri arasındaki sıra ve sütun gösterimlerinin önem sıralamalarının eş zamanlı olarak yapılamadığı durumlarda kullanılan bir yöntemdir (Özdamar, 2010).

Bu çalışmanın sonucunda küçükbaş hayvancılık ile ilgili elde edilen bulgulara göre yerli koyun ırkı varlığı Marmara Bölgesinde yer alan tüm iller için Merinos koyunu ve keçilerin varlığından daha yüksek bulunmuştur. Bunun yanında Merinos koyununun potansiyelinin keçilerden daha yüksek bulunduğu iller ise Balıkesir, Bursa, Edirne ve Tekirdağ'dır. Uyum analizi çalışmasının sonucunda ortaya çıkan bölgedeki Merinos ve melezlerinin oranının ülkemizdeki hayvan varlığındaki orandan fazla olması, bölgenin koyun yetiştiriciliği bakımından kültür ırkına daha fazla önem verdiğini göstermektedir. Keçi popülasyonundaki durum ise bölgede keçi yetiştiriciliğinin koyuna oranla düşük düzeyde olduğunu göstermiştir. Yalova ilindeki özel durum ilde kendine has kıvırcık koyunundan köken alan Yalova koyununun daha çok tercih edilmesiyle açıklanabilir. Bölgenin tamamında yerli koyun popülasyonunun Merinos ve keçilerden fazla bulunması ülke geneli ile uyumludur. Merinos koyunu, Cumhuriyet döneminde yapılan ıslah çalışmalarında Karacabey Merinosu ırkının ismini Bursa'nın Karacabey İlçesinden alması ve bu bölgede oluşturulmuş olması nedeniyle Bursa ve çevre illerde yüksek düzeyde tespit edilmiştir (Atav ve Buğdaycı, 2022).

Balıkesir ili kuzu üretimi bakımından coğrafi işarete sahiptir (Balıkesir Ticaret Borsası, 314 menşe, 2018 Türk Patent ve Marka Kurumu) ve burada üretilen kuzular da diğer bölgelere nazaran daha çok tercih edilmektedir. Marmara bölgesinde yapılan bu çalışmada da Balıkesir ilinin katkısı diğer illerden yüksek bulunmuştur. Benzer durum Çanakkale ilinde keçi yetiştiriciliği bakımından karşımıza çıkmaktadır. Çanakkale'de keçi peynirinin Ezine başta olmak üzere il genelinde yaygın olması, küçükbaş üretiminin keçinin lehine olmasını sağlamaktadır (Ilgar, 2019).

Büyükbaş hayvancılık ile elde edilen sonuçlara göre kültür ve kültür melezi sığır potansiyeli incelenen tüm illerde yerli sığır ırkı ve manda potansiyelinden oldukça yüksek bulunmuştur. Manda varlığının yerli ırk sığır varlığından yüksek olduğu iller sadece üç tane olup bunlar Kırklareli, Tekirdağ ve İstanbul'dur. Yerli sığır ırkı varlığı açısından Balıkesir'i sırası ile Çanakkale ve Bursa illeri izlemektedir. Kültür ırkı sığır yetiştiriciliğinde ise Balıkesir'den sonra sırası ile Bursa ve Çanakkale illeri gelmektedir. Manda yetiştiriciliği açısından ise İstanbul'un 2022 yılında Marmara Bölgesi'nde en önde gelen il olduğu görülmektedir (%40,6).

Büyükbaş yetiştiriciliğinde durum küçükbaşın tersi şeklindedir. Yerli ırk sığır yetiştiriciliğinde süt veriminin düşük olması tüketim bakımından ise inek sütünün daha yüksek oranda olması ülkemizde ve bu bölgede son 30 yılda yerli ırkların azalıp kültür ve melezlerinin artmasına neden olmuştur. Bölgede ülkemizin en fazla insan popülasyonuna

sahip ili olan İstanbul'un varlığı ve hayvansal ürün talebi tüm sektöre yansımıştır (TÜİK).

Uyum analizi sonucunda küçükbaş hayvancılık için yapılan hesaplamalara göre ilk iki boyutun birlikte varyansı açıklama oranı %11,7'dir. Büyükbaş hayvancılık için ise bu değer %7,1 olarak bulunmuştur. Her iki değer de oldukça düşük bulunmasına karşılık uyum analizi Marmara bölgesinde yetiştirilen hem küçükbaş hem de büyükbaş hayvan ırklarının iller arasında farklılık gösterdiğini ortaya koymuştur.

Küçükbaş hayvanlar için elde edilen grafiğe göre (Şekil 1) Bursa, Balıkesir ve Tekirdağ'ın Merinos koyunu yetiştiriciliği, Kocaeli, Kırklareli ve Sakarya'nın yerli ve diğer ırk koyun yetiştiriciliği ve Çanakkale'nin ise keçi yetiştiriciliğinde Marmara bölgesindeki küçükbaş hayvancılık sektörüne önemli katkı sağladığı söylenebilir. Büyükbaş hayvanlar için elde edilen grafikte ise (Şekil 2) Bursa, Balıkesir ve Sakarya'nın kültür sığırı yetiştiriciliği konusunda bölgeye önemli katkı sağladığı görülmektedir. Manda yetiştiriciliğinde ise en büyük potansiyele sahip ilin İstanbul olduğu görünmektedir. Grafikte bazı noktaların orijinden uzakta konumlandığı görünmektedir. Bu durum, ilgili değişkenlerin kategorilerine ait marjinal frekansların diğerlerine göre daha az olduğunu göstermektedir.

Bu çalışmada da kullanılan basit uyum analizi yöntemi ile iki boyutlu bir kontenjans tablosundan yararlanarak, tablonun "haritasını" çizmek amaçlanır. Uygulamada toplam inertianın yüksek olması arzu edilir. Bu değerin yüksek olması ele alınan değişkenler arasındaki ilişkinin yüksek olması anlamına gelmektedir (Uzgören, 2007). Bu çalışmada toplam inertianın düşük olmasının, özellikle büyükbaş hayvanlara ilişkin grafikte illere göre kümelenmede açık bir ayrımın yapılmasını kısmen de olsa engellediği düşünülmektedir.

Sıra ve sütun sayısının çok fazla olduğu ve gözlem sayısının yetersiz olduğu durumlarda tercih edilen bu yöntem bu çalışmada Marmara Bölgesi'nde yer alan on bir il için hayvan varlığını değerlendirmede tercih edilmiştir. Bu yöntemin hayvansal çalışmalarda kullanıldığı örnek çalışmalar literatürde mevcuttur. Abacı ve ark. (2020), kafes pozisyonu ile yumurtlama zamanı arasındaki ilişkiyi uyum analizi ile değerlendirmişlerdir. Şimşek ve Akçay (2021), uyum analizini kullanarak hayvan hastanesine getirilen hayvanların özelliklerini sekiz farklı değişken yönünden incelemişlerdir. Başpınar ve Mendeş (2000), PMSG dozu ile çeşitli doğum tipinde doğan kuzuların bir aylık yaşa kadar ölenlerin sayılarına ilişkin değerlendirmeyi basit uyum analizi ile yapmışlardır. Demir (2020) ise; çalışmasında Doğu Anadolu bölgesinde bulunan illere göre hayvan varlıklarını incelemiş, çalışmasında hayvanları özellikle kırmızı et yönünden ele alarak sığır, koyun ve keçi olarak kategorize etmiştir. Bu çalışmada ise değerlendirme, Marmara Bölgesi'ndeki hayvan varlığı açısından hem büyükbaş hem de küçükbaş hayvanlar için ayrı ayrı gerçekleştirilmiştir.

Hayvancılık tüm dünyada özellikle de gelişmiş ülkelerde ekonominin ayrılmaz bir parçasıdır. Türkiye'de de hayvancılık genel ekonomi içerisinde önemli bir yere sahip olup ulusal düzeyde geliştirilmesi gereken stratejik bir sektördür. Hayvancılık alanında yapılan araştırmaların yanı sıra elde edilen bulguların detaylı bir şekilde incelenerek iyileşme ve gelişmeyi sağlayacak önlemler alınması oldukça önemlidir. Bu aşamada kategorik verilerin sıklıkla elde edildiği hayvancılık ile ilgili çalışmalarda uyum analizinin kullanılması ve bu yöntemin karmaşık çapraz tabloların grafikler yardımıyla yorumlanması için önerilebilir. Bu çalışmanın sonuçları Marmara Bölgesi'nde bulunan illerdeki hayvan türlerinin varlığının incelenmesi ve hayvancılık ile ilgili yapılacak planlamada bu bilgilerin kullanılması bölgede hayvancılığın gelişmesi açısından oldukça önemlidir.

Çıkar çatışması

Yazarlar bu yazı için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan etmişlerdir.

Etik izin bilgisi

Bu çalışma "Hayvan Deneyleri Etik Kurullarının Çalışma Usul ve Esaslarına Dair Yönetmelik" Madde 8 (k) gereği HADYEK iznine tabi değildir.

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Benzerlik oranı

Makalenin benzerlik oranının sisteme yüklenen raporda belirtildiği gibi % 12 olduğunu beyan ederim.

Yazar katkıları:

Fikir/Kavram: EU, HÜ
Tasarım: EU, HÜ
Denetleme/Danışmanlık: EU, HÜ
Veri Toplama ve/veya İşleme: EU
Analiz ve/veya Yorum: EU
Kaynak Taraması: EU
Makalenin Yazımı: EU, HÜ
Eleştirel İnceleme: EU, HÜ

Kaynaklar

Abacı SH, Bayyurt L, Tahtalı Y, Şekeroğlu A, Duman M, 2020: Determination of relationship between cage position and laying time by correspondence analysis. *TURJAF*, 8(5), 1211–1215.

Aktürk D, 2004: Çoklu uyum analizi tekniğinin sosyal bilim araştırmalarında kullanımı. *Tarım Bilimleri Dergisi*, 10(2): 218-221.

Alpar R, 2011: Uygulamalı Çok değişkenli İstatistiksel Yöntemler, 355-382, Detay Yayıncılık, Ankara.

Atav R, Buğdaycı B, 2022: Türkiye'de kaliteli yapağı verimine sahip koyun ırkı eldesinde Merinoslaştırma faaliyetlerinin geçmişi, bugünü ve geleceğine genel bakış ve Türk merinosu (Karacabey merinosu) ırkının yapağı özelliklerine ilişkin önceki çalışmalar, *Tekstil ve Mühendis*, 29(127): 185- 197.

Başpınar E, Mendeş M, 2000: İki yönlü tablolarda uyum analizi tekniğinin kullanımı. *Tarım Bilimleri Dergisi*, 6(2): 98-106.

Demir Y, 2020: Doğu anadolu bölgesi illerine ait hayvan varlıklarının basit uyum analizi ile incelenmesi. *JIST*, 10(3), 2252-2259.

Ergün OF, Bayram B, 2021: Türkiye'de hayvancılık sektöründe yaşanan değişimler. *Bahri Dağdaş Hayvancılık Araştırma Dergisi*, 10(2), 158-175.

Gün Z, Çavuş Erdem Z, 2014: Assessing the factors affecting the mathematics success via correspondence analysis method. *AUJES*, 4(2), 98-118.

Ilgar R, 2019: Gıda kültürünün yansıması: Ezine peynirinin Türkiye ekonomisindeki yeri ve önemi. *Doğu Coğrafya Dergisi*, 24(41): 91-106.

Özdamar K. 2010: Paket programlar ile istatistiksel veri analizi, 445-482, Kaan Kitabevi, Eskişehir.

Polat M, 2017: Hayvancılık sektörünün TRA2 bölgesinin ekonomik kalkınması üzerine etkileri. *IJSSER*, 3(2), 631-643.

Suner A, Çelikoğlu C, 2008: Uygunluk analizinin benzer çok değişkenli analiz yöntemleri ile karşılaştırılması. *İstatistikçiler Dergisi: İstatistik ve Aktüerya*, 1 (1), 9-15.

Şimşek A, Akçay A, 2021: Erciyes Üniversitesi Veteriner Fakültesi Eğitim, Araştırma ve Uygulama Hastanesi'ne getirilen hayvanların uyum analizi ile değerlendirilmesi. *Erciyes Üniv Vet Fak Derg*, 18 (3), 182-189.

Türkiye İstatistik Kurumu, https://biruni.tuik.gov.tr/medas/-?kn=101&locale=tr, Erişim tarihi; 10.05.2023.

Türkiye İstatistik Kurumu, Nüfus kayıt sistemi, https://data.tuik.gov.tr/Bulten/Index?p=49685, Erişim tarihi; 10.11.2023.

Uzgören N, 2007: Uyum analizinin teorik esasları ve regresyon analiz ile benzerliğinin grafiksel boyutta karşılaştırılması. *Dumlupınar Üniversitesi Sosyal Bilimler Dergisi*, 18, 1-20.

Vural H, Fidan H, 2007: Türkiye'de hayvansal üretim ve hayvancılık işletmelerinin özellikleri. *Tarım Ekonomisi Dergisi*, 13(2): 49-59.

Yılmaz V, Saraçlı S, 2007: Çocuklarda suç türü ve nedenlerinin uyum analiziyle incelenmesi. *AKU-FEMÜBİD,* 7(1): 381-394.

HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Retrospective Evaluation of Dental and Gingival Health in 150 Dogs

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Abstract: The aim of this study was to calculate the prevalence of gingivitis, calculus, and traumatic dentoalveolar injury as a result of dental and gingival examinations of 150 dogs brought to Burdur Mehmet Akif Ersoy University Animal Hospital due to various health problems. In this study, dogs were examined by inspection without anaesthesia or invasive techniques. As a result of the examination, calculus (87.2%), gingivitis (60.7%) and traumatic dentoalveolar injuries (51.3%) were found in the majority of patients at rates higher than the reported prevalence values. It was emphasised that if existing diseases are not treated, the general health of the patient may be adversely and irreversibly affected.

Keywords: *Calculus, Dog, Gingivitis, Oral examination, Traumatic dentoalveolar injuries.*

150 Köpekte Retrospektif İnspektif Diş ve Gingiva Sağlığı Değerlendirilmesi

Özet: Bu çalışmada Burdur Mehmet Akif Ersoy Üniversitesi Hayvan Hastanesine çeşitli sağlık problemlerinden dolayı getirilen 150 köpeğin inspektif olarak diş ve gingiva yönünden gerçekleştirilen muayeneleri sonucunda gingivitis, kalkulus ve travmatik dentoalveoler yaralanma prevalanslarının hesaplanması amaçlanmıştır. Bu çalışmada, köpekler anestesi ve invazif teknikler kullanılmadan, inspeksiyon ile muayene edilmiştir. Muayene sonucunda hastaların çoğunluğunda, bildirilen prevalans değerlerinden daha yüksek oranlarda kalkulus (%87.2), gingivitis (%60.7) ve travmatik dentoalveoler yaralanmalar (%51.3) ile karşılaşılmıştır. Mevcut hastalıkların tedavi edilmediği takdirde ise hastanın genel sağlığının olumsuz ve geri dönülemez bir şekilde etkilenebileceği vurgulanmıştır.

Anahtar Kelimeler: *Gingivitis, Kalkulus, Köpek, Oral muayene, Travmatik dentoalveoler yaralanmalar.*

Introduction

Dentistry is one of the most overlooked and undervalued veterinary medicine services yet one of the most profitable (Lengyel, 2018). Practical assessments of oral health include gingival examination, calculus examination, and detection of traumatic dentoalveolar injuries (TDI) (Asproni et al., 2022; Soukup et al., 2015).

Traumatic dentoalveolar injuries include injuries to the supporting structures of the tooth, such as the tooth, alveolar bone, or periodontal ligament, caused by a direct force (Bilyard and Juriga, 2021; Soukup et al., 2015). TDIs can occur in situations such as vehicle accidents, blunt force trauma, falls, or masticatory activities. However, despite the suspected high prevalence, studies of TDI in animals have been historically neglected and studies of the epidemiology of TDI in dogs are limited; therefore, further research in this area is warranted (Capik et al., 2000; Mulherin et al., 2013; Soukup et al., 2015). When the pulp is exposed, it is referred to as a complex fracture and is associated with caries erosion. In such cases, the spread of pathogenic bacteria into the pulp canal or periapical region can lead to abscess formation. Complex fractures can result in pain, pulpitis, and pulpal necrosis (Soukup, 2018). If the infection progresses to the apical end of the root canal, it can lead to osteomyelitis and loss of surrounding bone (Holmstrom, 2019).

Malocclusion is defined as the misalignment of teeth in the dental arches when the mouth is closed (Bellows, 2022). Malocclusion can be caused by skeletal malocclusion, which is seen as an inconsistency in the length and/or width of the jaw; dental malposition, which refers to the abnormal placement of the teeth relative to each other or their position in the mouth; or a combination of both (Milella, 2015). Occlusion evaluation included assessment of cranial morphology, symmetry, and jaw length during the examination. The position of the teeth was then assessed by lifting the labium. The teeth were then checked for trauma and the oral soft tissues were checked for abnormalities (Southerden, 2017). Examination of the canines and premolars provides a good guide for assessing the occlusion and also helps to determine whether the malocclusion is due to skeletal or dental causes (Milella, 2015).

In malocclusion class 1, if the tooth is in an abnormallingual position, it can lead to ulceration and perforation of the palatum durum (Holmstrom, 2019). In malocclusion class 2, if left untreated, this malocclusion can lead to oronasal fistula formation in severe cases (Southerden, 2017). In Class 3 malocclusion, misalignment of the maxillary and mandibular premolars can be seen. This can cause trauma to the teeth, to wear and tear. In Class 4 malocclusion, irregular occlusion results in the mandibular canine teeth striking the maxillary palatum, gingiva, or labium. This can lead to periodontal disease, oronasal fistulae, traumatic pulpitis, and severe pain and infection, depending on the location of trauma. Traumatic pulpitis can lead to endodontic diseases or abscesses (Niemiec, 2010). Linguoversion of the mandibular canines causes obstruction of the crown tips by the palatal mucosa (Niemiec et al., 2017).

Periodontal disease is one of the most common diseases in veterinary clinical practice, affecting 80% of dogs over two years of age (Barbosa et al., 2023). The clinical inflammatory state of the periodontal tissues can be divided into two categories: gingivitis and periodontitis. Gingivitis is characterised by erythematous and swollen gingiva (Asproni et al., 2022). Periodontitis is a severe form of periodontal disease in which the periodontium is affected. Gingivitis occurs before periodontitis, but does not always lead to periodontitis (Reiter, 2018).

Periodontal disease begins with the accumulation of plaque on the tooth surface (Holcombe and Wallis, 2020). Within seconds of brushing, the dental pellicle begins to accumulate and within hours, gram-positive bacteria colonise the pellicle and begin to form a plaque biofilm. With the addition of bacterial by-products, plaque changes colour from ivory to yellow and greyish. Halitosis is caused by volatile sulfur compounds produced by oral bacteria. Mineralisation of the plaque biofilm leads to the formation of tartar. (Dodd & Lobprise, 2019). The color of the calcified part of the tooth crown can vary from dark yellow to dark brown (Lobprise 2021). Dental calculus causes irreversible damage to the tissues that support the teeth, such as the gingiva, alveolar bone, cementum, and periodontal ligament and also, as tartar progresses, tooth mobility and loss occur (Chain et al., 2020).

Chronic periodontitis can cause osteomyelitis of the maxilla or mandible. Tooth dislocation may occur due to periodontal disease, and the tooth may extrude from the alveolus (Niemiec et al., 2017). In geriatric and small-breed dogs, this can lead to iatrogenic fractures during tooth extraction. As the disease progresses, an oronasal fistula formation can also be observed (Marretta and McFadden, 2013). Bacteria involved in periodontal disease can migrate to other parts of the body with bacteremia and cause endocarditis, nephritis, and hepatitis by colonising the relevant sites (Bourguignon et al., 2012).

The aim of this study was to demonstrate that, in dogs examined retrospectively, the lack of control over dental and gingival health results in higher disease rates than the reported prevalence by other studies, and that the current situation may worsen as oral pathologies progress and reach irreversible points.

Materials and Methods

The number of patients in the study consisted of 150 dogs brought to Burdur Mehmet Akif Ersoy University Animal Hospital for various health problems between January-June 2023. The teeth and gingiva were examined by only inspection in order to examine without anaesthesia and scored for gingival index, calculus index and visual traumatic dentoalveolar injury. TDIs were classified as complicated, uncomplicated, or luxated TDIs based on the classification used by Soukup et al. (2015) (Figure 1) (Figure 2). To obtain diagnostic radiographs of the canine dentition, patients must

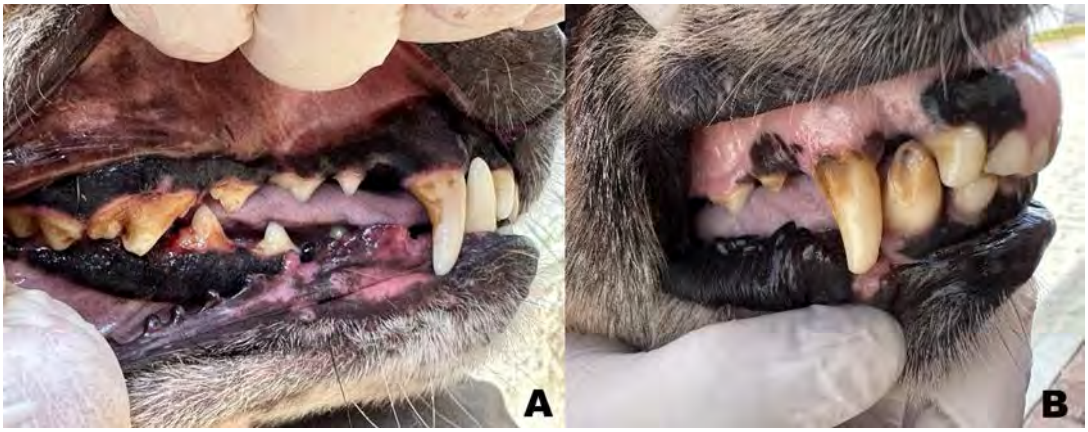


Figure 1. (A) Calculus 3.degree with 3.degree gingival index, also there is a uncomplicated fracture at 4.premolar tooth (108). (B) Complicated fracture at mandibular canine tooth (404).

Figure 2. (A) Lateral view of the teeth of a 4-year-old female dog with a gingival index of 2 and a calculus index of 2. In addition to gingival inflammation, the calculus covers 1/3-2/3 of the buccal tooth surface with minimal subgingival deposition. (B) Complicated fractured canine in a 3-year-old female dog.

be under general anesthesia (Bannon,2013). Owing to this fact, this study recorded data that could only be seen by inspection. (Figure 1) (Figure 2).

This work is not subject to HADYEK authorization under Article 8 (k) of the "Regulation on the Practice and Basics of Animal Experiment Ethics Boards".

Malocclusion: The angle classification system we used has been adapted from human dentistry to categorise veterinary dental occlusion by Bellows (2022) for felines. Class 0 represents a normal occlusion (Bellows, 2022). Class 1 shows a normal jaw relationship and the abnormal positioning of one or more teeth. Class 2 malocclusion are skeletal malocclusion in which the mandible is relatively shorter and usually narrower than the maxilla, so the teeth do not close normally (Southerden, 2017). It is considered to be an inherited trait unless it is the result of trauma (Dodd and Lobprise, 2019). Class 3 is a skeletal malocclusion with a long mandible or short maxilla. Class 4 is a special classification of mandibular and maxillary asymmetry, in which class 0 or 2 is seen in one mandible and class 3 in the other maxilla (Bellows, 2022).

Gingival Index: Gingivitis scoring we used in this study, also known as the gingival index, has been used to assess gingival health (Niemiec, 2010). A gingival index score of zero meant that there was no inflammation of the gingiva. In grade one, the gingiva showed mild inflammation and slight changes in texture and colour. In the second degree, moderate inflammation, redness, oedema, hypertrophy and bleeding occur when pressure is applied to the gingiva. In the third degree, severe redness and hypertrophy of the gingiva is observed and spontaneous bleeding may occur when pressure is applied (Löe and Silness, 1963).

Calculus Scoring: In calculus scoring we used, at grade zero shows no calculus is seen on the tooth surface, at grade one shows the supragingival calculus covering <1/3 of the buccal tooth surface. In the second degree, a moderate calculus is seen, covering 1/3-2/3 of the buccal tooth surface with minimal subgingival deposition (Fig. 2.A). In the third

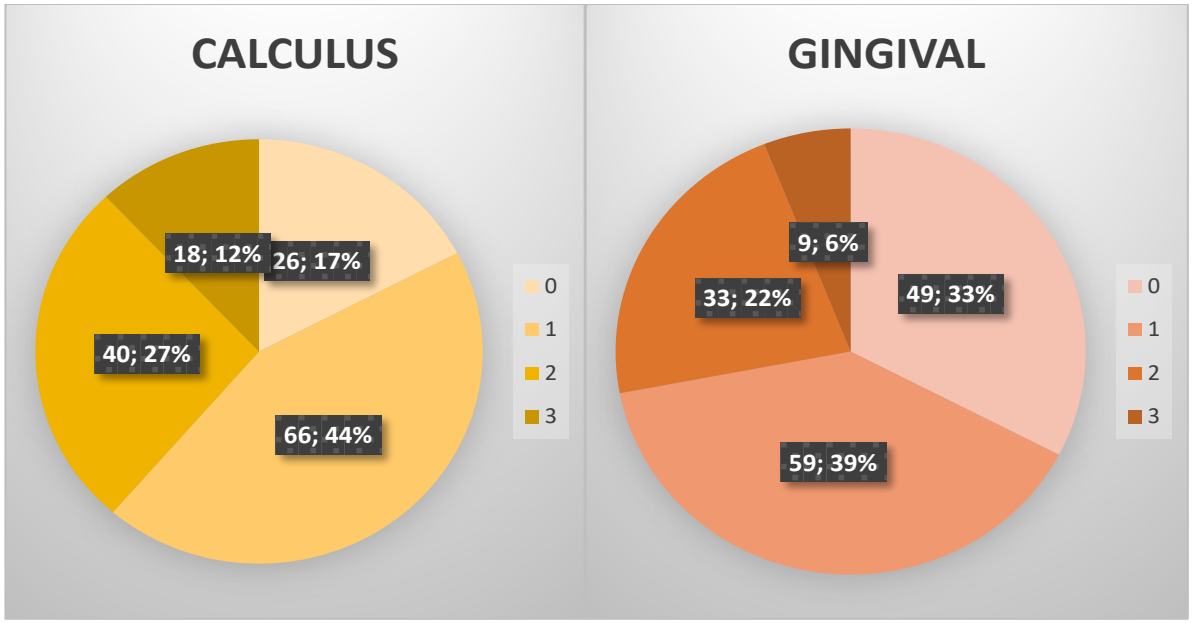
degree, there is a subgingival accumulation of calculus covering more than 2/3 of the buccal tooth surface (Greene and Vermillion, 1964).

Results

Gingival Index: The number of dogs with a gingival index of 1 was 59 (39.3%), 33 (22%) with a gingival index of 2 and 9 (6%) with a gingival index of 3. After examination of 150 dogs, 49 (32.6%) dogs had gingival index 0. According to the data obtained, gingivitis was found in 101 (60.7%)

Calculus Index: Calculus index was scored as 1 in 66 (44%), 2 in 40 (26.6%) and 3 in 18 (12%) of 150 dogs in addition, 26 (17.3%) dogs had calculus index 0. According to the data obtained, calculus in 124 (87.2%) of the 150 dogs examined (Table 1).

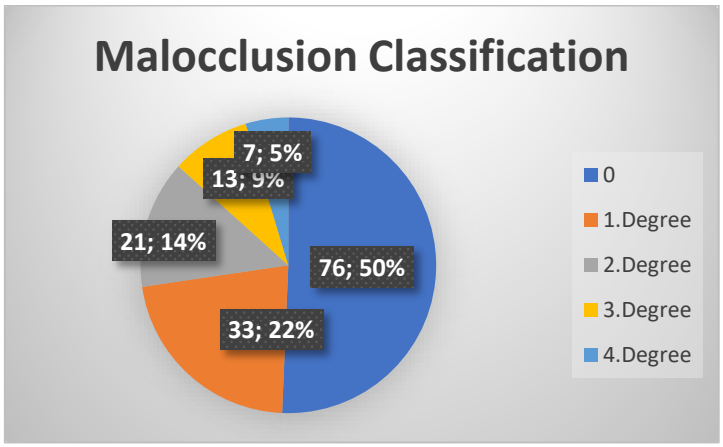
Table 1. The gingival and calculus indices and percentages of 150 dogs examined by the inspection method are shown in the figure.



The numbers indicate the calculus degrees on the left and the gingival index degrees in the right.
0 represents, 0 degrees, 1 represents 1. degrees, 2 represents 2. degrees, and 3 represents 3. degrees.

Malocclusion Classification: As a result of the examinations, 33 (22%) dogs had grade 1 malocclusion, 21 (%14) dogs had grade 2.13 (8%) dogs had grade 3 malocclusion and 7 dogs had grade 4 malocclusion. A total of 53 dogs were found to have malocclusions, of which 33 (43.4%) were 1st degree malocclusions, 21 (27.6%) were 2nd, 13 (%17.1) were 3rd and 7 (9.2%) were 4th degree malocclusions. In addition, 76 (%50.6) dogs had an occlusion classification 0. (Table 2).

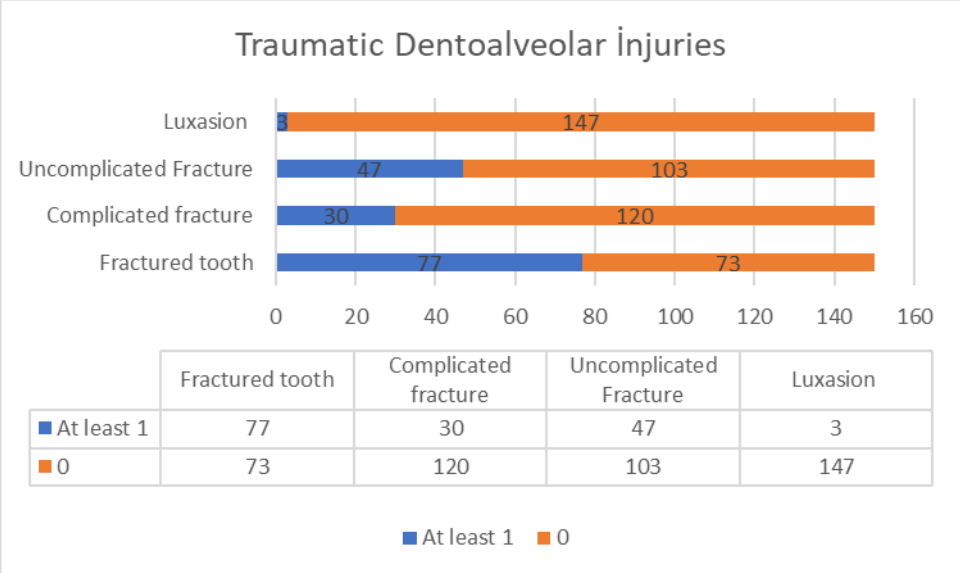
Table 2. Pie chart of malocclusion classification of dogs.



The maloclusion types of 150 dogs examined on the left are shown in the pie chart.
On the right is the color description of the charts slices.

Traumatic Dentoalveolar Injury: As a result of the prospective visual examination, 77 (51.3%) of 150 dogs had at least 1 tooth fracture and 30 (38.9%) of these dogs had complex fractures. In total, 30 of 150 dogs (20%) had at least 1 complicated fracture. Dogs with only uncomplicated fractures accounted for 61% of the dogs with at least one fracture and represented 47 (31.3%) of the total 150 dogs studied. In addition, 3 (2%) of the 150 dogs were found to have tooth dislocation due to trauma (Table 3).

Table 3. Examination results of dogs in terms of TDI.



The graph above shows the percentage distribution and number of 150 dogs with traumatic dentoalveolar injuries.

Discussion and Conclusions

In this study, dogs presented to Burdur Mehmet Akif Ersoy University Animal Hospital for variety reasons were examined. Most of the dogs examined had calculus and gingivitis, 51.3% had TDI, and 35% had occlusal disorder.

In general, TDIs have been reported to affect 26.2% of dogs (Soukup et al., 2015). In contrast, according to our study, 77 dogs (51.3%) had at least one TDI. Treatment of uncomplicated tooth fractures includes closure of all exposed dentin tubules as soon as possible. Annual radiographs are recommended to monitor changes in tooth colour, periapical disease and enlarged root canals (Juriga and Bilyard, 2021). In accordance with this information, the owners of patients with pathology were informed of the treatment. According to the data obtained in this study, 20% of the patients examined had complicated fractures. It was found that complicated fractures lead to pain, pulpitis and pulp necrosis over time (Soukup, 2018). It has been reported that due to exposure of the pulp, a coronal fracture or caries erosion can allow pathogenic bacteria to enter the pulp canal in a short period of time or cause a periapical abscess within the canal or with the spread of infection, and it has been stated that the progression of infection to the apical end of the root canal can lead to osteomyelitis and loss of surrounding bone (Marretta and McFadden, 2013). The owners of the dogs with complicated fractures were informed about the treatment and advised to follow the treatment path as soon as possible.

It has been emphasised that dislocation injuries result in pulp necrosis, that teeth are susceptible to root resorption or infection if left untreated, and that all teeth with dislocation injuries should be re-evaluated with follow-up radiographs between 6 and 12 months, in addition to the time between the accident and treatment which may be critical for a positive outcome (Bilyard and Juriga, 2021; Startup, 2010). In our study, it was observed that 2% of the dogs examined had dislocated teeth (Figure 3.B). Patients with dislocations were started to be treated with the consent of their owners.

Berman et al. (2023) reported the presence of malocclusion 1 variant in 65% which is nearly 20% higher than this study. Their study has similar number for malocclusion 2 with 30%. When it comes to malocclusion 3 there is a significant difference of almost 20%, the number of dogs we saw malocclusion 3 is less than theirs. Their number for Malocclusion 4 is 5.6% while our number is 9.2% (Figure 3.A).

According to Holcombe and Wallis (2020), an average prevalence of 9.3-18.2% has been reported for periodontal disease in canine populations. A much higher prevalence of 44-100% has been reported in detailed examinations of anaesthetized dogs (Holcombe and Wallis, 2020). The gingival index of the dogs examined was 60.7% and calculus was found in 87.2% in our study, which are the main reason of the periodontal disease.

In conclusion, according to the data obtained by evaluating the gingivitis index, the calculus index and the traumatic dentoalveolar injuries, it was concluded that

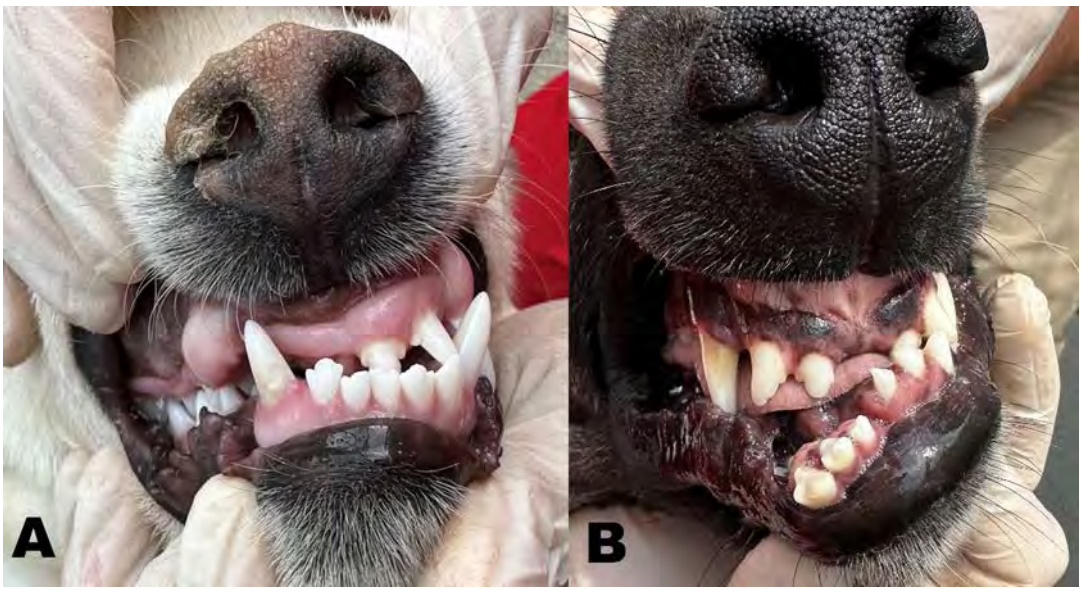


Figure 3. (A) Rostral view of a dog with a grade 4 malocclusion. In addition to mandibular and maxillary asymmetry, a grade 3 malocclusion is also noted. (B) Rostral view of a dog with lateral luxation. Teeth 401, 402 and 403 are dislocated, breaking the alveolar bone.

dental and gingival examinations in dogs should be performed regularly by the veterinarians in Turkey. The numbers this study found are generally higher compare to other studies around the world. The necessary information and advice should be given to the dog owners to protect the oral health of their dogs, the course of the current situation may be negatively affected if the detected diseases are not followed up and if they are not treated, they may develop into irreversible situations that may affect the life of the patients.

Conflict of Interest

The authors have stated that there is no real, potential or perceived conflict of interest for this article.

Ethical Approval

This work is not subject to HADYEK authorization under Article 8 (k) of the "Regulation on the Practice and Basics of Animal Experiment Ethics Boards"

Similarity Rate

We state that the similarity rate of the article is 11% as stated in the report uploaded to the system.

Author Contributions

Motivation / Concept: ÖŞŞ, MAK, FCO
Design: MAK, FCO
Control/Supervision: ÖŞŞ
Data Collection and / or Processing: MAK, FCO
Analysis and Interpretation: MAK, FCO
Literature Review: MAK, FCO
Writing the Article: ÖŞŞ

References

Asproni P, Cozzi A, Fonseca MJ, Lafont-Lecuelle C, de Oliveira J, Pageat P, Palmeira I, 2022: Dental Pain in Cats: A Prospective 6-Month Study. *J Vet Dent,* 39(4), 369-375.

Barbosa, E, Hauptli L, Pires PGS, Moraes P, 2023: Strategies to improve the home care of periodontal disease in dogs: A systematic review. *Res Vet Sci*, 154, 8-14.

Barnvos D, Harvey C, Serfilippi L, 2015: Effect of frequency of brushing teeth on plaque and calculus accumulation, and gingivitis in dogs. *J Vet Dent,* 32(1), 16-21.

Bellows J, 2022: Occlusal Disorders In: Feline Dentistry, Bellows J (Ed), 410-432, John Wiley and Sons, USA.

Berman M, Scanlan AJF, Soltero-Rivera M, 2023: Prevalence of Dental and Skeletal Malocclusions in Mesaticephalic and Dolichocephalic Dogs-a Retrospective Study (2015-2018). *J Vet Dent, 40*(2), 143-153.

Bilyard K, Juriga S, 2021: Working Dog Dentistry. Veterinary Clinics. *J Small Anim Pract,* 51(4), 779-802.

Bourguignon E, Daibert APF, Moreira MAS, Pieri FA, 2012: Periodontal Disease in Dogs In: A Bird's-Eye View of Veterinary Medicine, Perez-Marin CC (Ed), 119-140, InTech, Croatia.

Capik I, Ledecký V, Ševčík A, 2000: Tooth fracture evaluation and endodontic treatment in dogs. *Acta Vet Brno,* 69(2), 115-122.

Chain CP, Lehr W, Pignone VN, Pinto CFD, Trevizan L, 2020: Evaluation of teeth injuries in Beagle dogs caused by autoclaved beef bones used as a chewing item to remove dental calculus. *PLoS One,* 15(2), 1-15.

Daculsi G, Hamel L, Le Brech C, Le Nihouannen JC, 1996: Epidemiologic study of canine teeth fractures in military dogs. *J Vet Dent,* 5(1), 10-18.

Dodd JRB, Lobprise HB, 2019: Oral anatomy and physiology In: Wiggs's veterinary dentistry principles and practice, Lobprise HB, Dodd JRB (Eds), 1-24, John Wiley and Sons, USA.

Gawor J, Niemiec BA, 2014: Oral pathology in dog and cat: overview of selected problems. *Vet nurs*, *5*(1), 10-18.

Greene, JG, Vermillion JR, 1964: The simplified oral hygiene index. *J Am Dent Assoc,* 68(1), 7-13.

Hetzel S, Paul A, Soukup J, 2015: Classification and Epidemiology of Traumatic Dentoalveolar Injuries in Dogs and Cats: 959

Injuries in 660 Patient Visits (2004-2012). *J Vet Dent,* 32(1), 6–14.

Holcombe LJ, Wallis C, 2020: A review of the frequency and impact of periodontal disease in dogs. *J Small Anim Pract,* 61(9), 529-540.

Holmstrom SE, 2019: The Oral Examination and Disease Recognition In: Veterinary Dentistry A Team Approach, Holmstrom SE (Ed), 21-72, Elsevier Health Sciences, Missouri.

Lengyel K, 2018: Why'Dental'Is a Dirty Word. *J Am Vet Med Assoc, 3*(4).

Lobprise HB, 2021: Intraoral Radiology and Advanced Imaging In: Blackwell's five-minute veterinary consult clinical companion small animal dentistry, Lobprise HB (Ed), 21-46, John Wiley and Sons, Texas.

Löe H, Silness J, 1963: Periodontal disease in pregnancy I. Prevalence and severity. *Acta Odontol Scand,* 21(6), 533-551.

Marretta SM, McFadden T, 2013: Consequences of untreated periodontal disease in dogs and cats. *J Vet Dent,* 30(4), 266-275.

Milella L, 2015: Occlusion and malocclusion in the cat: What's normal, what's not and when's the best time to intervene? *J Feline Med Surg,* 17(1), 5-20.

Mulherin BL, Snyder CJ, Soukup JW, 2013: Prevalence and nature of dentoalveolar injuries among patients with maxillofacial fractures. *J Small Anim Pract,* 54(1), 9-14.

Niemiec BA, 2017: Advanced and Future Options For Oral and Maxillofacial Imaging In: Practical Veterinary Dental Radiography, Niemiec BA (Ed), 347-358, CRC Press, New York.

Niemiec BA, 2010: Pathology in the pediatric patient In: A colour handbook of small animal dental and oral maxillofacial disease, Niemiec BA (Ed), 89-126, Manson Publishing Ltd, London.

Niemiec BA, 2010: Problems with the gingiva In: A colour handbook of small animal dental and oral maxillofacial disease, Niemiec BA (Ed), 159-181, Manson Publishing Ltd, London.

Reiter AM, 2018: Commonly encountered dental and oral pathologies In: BSAVA manual of canine and feline dentistry and oral surgery, Gracis M, Reiter AM (Eds), 89-118, British Small Animal Veterinary Association, Aberystwyth.

Southerden P, 2017: Malocclusion in dogs and cats. *Vet nurs*, 8(4), 207-214.

Startup S, 2010: Wire-composite splint for luxation of the maxillary canine tooth. *J Vet Dent,* 27(3), 198-202.

Soukup J, 2019: Traumatic Dentoalveolar Injuries In: Wiggs's veterinary dentistry principles and practice, Lobprise HB, Dodd JRB (Eds), 109-130, John Wiley and Sons, USA.

HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Açlık Stresine Maruz Kalan *Corynebacterium pseudotuberculosis'*in SDS-PAGE İle Proteinlerinin Analizi

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Abstract: *Corynebacterium pseudotuberculosis*, hücre içi bir patojen olup küçük ruminantlarda kazeöz lenfadenit (KLA) hastalığına sebep olan, Gram-pozitif fakültatif bir bakteridir. Ayrıca *C. pseudotuberculosis,* insanlarda ve hayvanlarda hastalık oluşturan bir bakteri türüdür. Bu bakteri, açlık stresine maruz kaldığında, hücresel yapısını ve metabolizmasını değiştirerek adaptasyon gösterir. Bu değişiklikler, bakterinin protein ekspresyonunda da değişikliklere neden olabilir.

Bu çalışmada, *C. pseudotuberculosis*'in açlık stresine maruz bırakıldığında ve genel besiyerlerinde üretildiğinde protein profillerinde meydana gelen farklılıkların araştırılması amaçlanmıştır. Daha önce izole edilmiş olan bir saha suşu ve referans olarak kullanılan *C. pseudotuberculosis* suşu Tryptic Soy Broth ve RPMI 1640 (Roswell Park Memorial Institute) besiyerlerinde üretildikten sonra tüm proteinleri SDS PAGE ile karşılaştırılarak incelendi.

Sonuç olarak, açlık strese maruz bırakılan ve bırakılmayan *C. pseudotuberculosis* suşları arasında protein yapıları arasında profil farklılıkları olduğu tespit edilmiştir.

Anahtar Kelimeler: *Corynebacterium pseudotuberculosis, RPMI, SDS-PAGE, Stres.*

Analysis of Proteins of *Corynebacterium pseudotuberculosis* Exposed to Starvation Stress by SDS-PAGE

Abstract: *Corynebacterium pseudotuberculosis* is a Gram-positive facultative bacterium that is an intracellular pathogen causing caseous lymphadenitis (CLA) in small ruminants. In addition, *C. pseudotuberculosis* is a bacterial species that causes disease in humans and animals. When exposed to starvation stress, this bacterium adapts by changing its cellular structure and metabolism. These changes may also result in alterations in the protein expression of the bacterium.

In this study, we aimed to investigate the differences in protein profiles of *C. pseudotuberculosis* when exposed to starvation stress and when grown on general media. A previously isolated field strain and a reference strain of *C. pseudotuberculosis* were grown in Tryptic Soy Broth and RPMI 1640 (Roswell Park Memorial Institute), and all proteins were analyzed by SDS PAGE.

As a result, it was determined that there were profile differences in protein structures between *C. pseudotuberculosis* strains exposed and not exposed to starvation stress.

Keywords: *Corynebacterium pseudotuberculosis, RPMI, SDS-PAGE, Stress.*

Giriş

C. pseudotuberculosis, Gram pozitif bir bakteri olup, hareketsiz, sporsuz, kapsülsüz, kokoid veya çomak morfolojisinde, fakültatif hücre içi bakteridir. Etken, aerobik veya fakültatif anaerobik ortamlarda, katı besi yerlerinde 37 ºC'de 48-96 saatte küçük, smooth tarzda yuvarlak, sarımsı veya grimsi-beyazımsı koloniler meydana getirmektedir. Proteolitik etkisi bulunmayıp, biyokimyasal aktivitesi zayıftır ve değişkenlik göstermektedir. Etken, daha çok koyun ve keçilerde kazeöz lenfadenitis (KLA)'in spesifik etkenidir. KLA, özellikle eksternal lenf yumruları (eksternal form) ve akciğerler başta olmak üzere birden fazla iç organda kapsüllü apseler meydana getiren (internal form) kronik bir enfeksiyon meydana getirir. Dünyanın birçok yerinde yaygın olarak görülen bu hastalıkta apse oluşumuyla beraber erken doğum, abortlar, deri ve yapağı kalitesinde bozulma, çok şiddetli zayıflamaya bağlı olarak et verimi ve kalitesinde düşme, özellikle genç hayvanlarda aşırı olmak üzere nadiren de olsa ölümler gözlenebilmektedir (Dorella ve ark., 2006; İlhan, 2001; Middleton ve ark., 1991; Parin ve ark., 2018).

Küçük ruminantlarda, *C. pseudotuberculosis* patogenezine katkıda bulunan virülans faktörleri ile ilgili olarak, 31.4 kDa'luk fosfolipaz D (PLD) bu bakterinin başlıca virülans faktörü olarak tanımlanmaktadır. PiCp1 operonundaki genler (*fagA, fagB, fagC, fagD)* tarafından oluşturulan virülans faktörlerinin açlık stresinde bakterinin eksprese ettiği genlerden bazılarıdır (Billington ve ark., 2002). Bu virülans faktörleri, *C. pseudotuberculosis*'in farelerde hastalık oluşturma yeteneği için gereklidir. FagA (37,5 kilo dalton), demir edinimi için gerekli olan bir heme-bağlayıcı proteindir. FagB (20 kDa), hücre duvarı stabilitesini sağlayan bir proteindir. FagC (25 kDa), fagositozdan kaçınma için gerekli olan bir proteindir. FagD (22 kDa), hücre içi hayatta kalma için gerekli olan bir proteindir. Ayrıca, bir ATP bağlayıcı sitoplazmik membran proteini (37,5 kDa) ve bir demir bağlayıcı siderofor CiuA (50 kDa) (Ribeiro ve ark., 2014) proteini bulunmaktadır.

Açlık stresinde protein düzeylerindeki değişikliklerden biri ATP bağlayıcı sitoplazmik membran proteini düzeyleri artmasıdır. Bu protein, bakterinin enerji üretimi için gereklidir. Açlık stresi altında, bakteri enerji tasarrufu sağlamak için ATP üretimlerini artırmaya çalışır. Bu, ATP bağlayıcı sitoplazmik membran proteini düzeylerinin artmasına neden olur ve demir bağlayıcı siderefor protein düzeyleri artar. Açlık stresi altında, bakteri demir eksikliği stresine maruz kaldığından bu da bakterinin demir alımını arttırmasına neden olur. Bu nedenle, demiri bağlayıcı siderefor proteini düzeyleri artar. Sonuçta bu değişikliklerin, bakterinin enerji üretimini artırmasına, demir alımını artırmasına ve fagositozdan kaçmasına yardımcı olduğuna inanılmaktadır (Lee ve ark., 2017).

C. pseudotuberculosis'in tüm izolatlarında bulunan ve SDS'de çözünen proteinlerin dört gruba ayrılabildiğini ve bunların; (i) 119 kDa'dan büyük olan yüksek moleküler kütleli proteinler, (ii) 84, 64 ve 58 kDa moleküler kütleli üç protein seti, (iii) 33 ile 30 kDa moleküler kütleli proteinlerden oluşan bir çift ve (iv) 25 ile 20 kDa düşük moleküler kütleli proteinler olduğunu bildirilmiştir (Egerton ve ark., 1997).

Bu çalışmada, *C. pseudotuberculosis*'in açlık stresine maruz bırakıldığında ve genel besiyerlerinde üretildiğinde protein profilleri arasındaki farklılıkların incelenmesi amaçlanmıştır.

Materyal ve Metot

***C. pseudotuberculosis* suşları**: Bir adet referans (ATCC 19410) ve daha önceden izole edilip suş bankasında saklanan bir saha izolatı olmak üzere 2 adet *C. pseudotuberculosis* suşu Dollvet Biyoteknoloji A.Ş.'den temin edilmiştir. Bu çalışma "Hayvan Deneyleri Etik Kurullarının Çalışma Usul ve Esasları Hakkında Yönetmelik"in 8(k) maddesi uyarınca HADYEK iznine tabi değildir.

***C. pseudotuberculosis* suşlarının doğrulanması:** Referans (ATCC 19410) ve izole edilen *C. pseudotuberculosis* suşlarının VİTEK/COMPACT VK2C22273 ile identifikasyonuları teyit edildikten sonra suşlara PZR yapıldı (forward primer PLD-F 5'-ATA AGC GTA AGC AGG GAG CA-3' / reverse primer PLD-R1 5'-ATC AGC GGT GAT TGT CTT CC-3) ve numunelerin *C. pseudotuberculosis* olduğu doğrulandı. Toplam 50 µL PCR karışımı; 5 µL PCR Buffer (10 mMTris–HCl, pH:9.0, 50 mMKCl, %0,1 Triton X-100), 5 µL 25 mM MgCl2, 250 mM dNTPs, 1 µL her bir primerden, 2 µL Taqpolimeraz ve 5 µL template DNA içerdi. Amplifikasyon aşaması; 95 °C'de 3 dakika ön denaturasyon, daha sonra 1 dakika 95 °C'de 1 dk, 58 °C'de 40 saniye, 68 °C'de 90 saniye olarak 40 döngü halinde yapıldı. Son aşamada da, 1 kez 68 °C'de 7 dakika final zincir uzaması ile amplifikasyon tamamlandı (Pacheco ve ark., 2007). PZR numuneleri yatay elektroforez'de %1.5'luk ethidium bromid içeren (0.5 mg/mL) agaroz jelde (70 Voltta, 50 dakika-1 saat aralığında) yürütüldü. Süre sonunda agaroz jel ultraviolet ışık altında gözlendi.

***C. pseudotuberculosis* suşlarının çoğaltılması:** Referans suş ve izolat, %5 koyun kanlı agarlara ekilerek %5 CO_2 'li inkübatörde 24 saat inkübe edildi. Süre sonunda üremesi görülen besiyerlerinden suşlar toplanarak içlerinde 5'şer ml Tryptic Soy Broth (TSB) ve Roswell Park Memorial Institute (RPMI) besiyeri bulunan 15 ml'lik falkonlara pasajı yapılarak %5 CO_2 'li inkübatörde 24 saat inkübe edildi. Süre sonunda yine içlerinde 9'ar ml Tryptic Soy Broth (TSB) ve RPMI besiyeri bulunan 15 ml'lik falkonlara 1'er ml alınarak 1/10 oranında pasajları yapılarak %5 CO_2 'li inkübatörde 24 saat inkübe edildi.

***Corynebacterium pseudotuberculosis* suşlarının SDS-PAGE ile analizi**: Üreyen bakteri kültürü 6000 rpm'de 10 dk santrifüj edildikten sonra üstteki süpernatant kısmı atılarak dipte kalan kültür, yükleme tamponu (Laemmli 2X buffer) (Sigma Lot #SLBT2607), 500mM Tris/HCl pH 6.8); %4 (w/v SDS; %10 (v/v gliserol; %10 (v/v) 2-β-merkaptoetanol ve %0.004 bromfenol mavisi) ile 1/3 oranında dilüe edilerek 5 dakika 95 ^{0}C'de kaynatıldı (Güllü Yücetepe, 2023; Laemmli, 1970).

Numunelerin yüklenmesi için, %12'lik ayırıcı (resolving) jel monomer homojenizasyonu hazırlandı ve hazırlanan jel karışımı mikropipet ile döküldü (4 ml saf su (dH_2O), 3,3 ml %30 Akrilamid/Bis-akrilamid, 2,5 ml 1,5 M Tris (pH: 8.8), 100

µl %10 SDS, 100 µl %10 Amonyum Persülfat ve 4 µl TEMED). Jel taksim edildikten sonra, yürütme jelinin üzerinde hava kabarcığı kalmaması amacıyla mikropipet yardımı ile distile su döküldü. Jelde polimerizasyonun sağlanması nedeniyle 20-30 dakika beklendi. Yürütme jeli polimerizasyonundan sonra aynı prosedür yükleme jeli için de yapıldı. Yükleme jelindeki polimerizasyondan emin olunduktan sonra %4'lük yükleme (stacking) jeli için monomer homojenizasyonu hazırlandı ve hazırlanan jel karışımı mikropipet ile döküldü (4 ml saf su (dH_2O), 3,3 ml %30 Akrilamid/Bis-akrilamid, 2,5 ml 1,5 M Tris (pH: 6.8), 100 µl %10 SDS, 100 µl %10 Amonyum Persülfat ve 4 µl TEMED) (Öztürk ve ark., 2023)

Hazırlanan yükleme jeli bekleme yapılmadan ayırıcı jelin üstüne eklendi ve camlara uygun kalınlıkta olan jel tarakları yerleştirildi. Jelde polimerizasyonun sağlanması nedeniyle 20-30 dakika beklendi. Yükleme jelindeki polimerizasyondan emin olunduktan sonra taraklar jel bozulmayacak şekilde çıkarıldı ve jel camlar ile birlikte elektroforez sistemine yerleştirildi. Marker (10 µl) ve numuneler (20 µl) jeldeki kuyucuklara yüklendi.

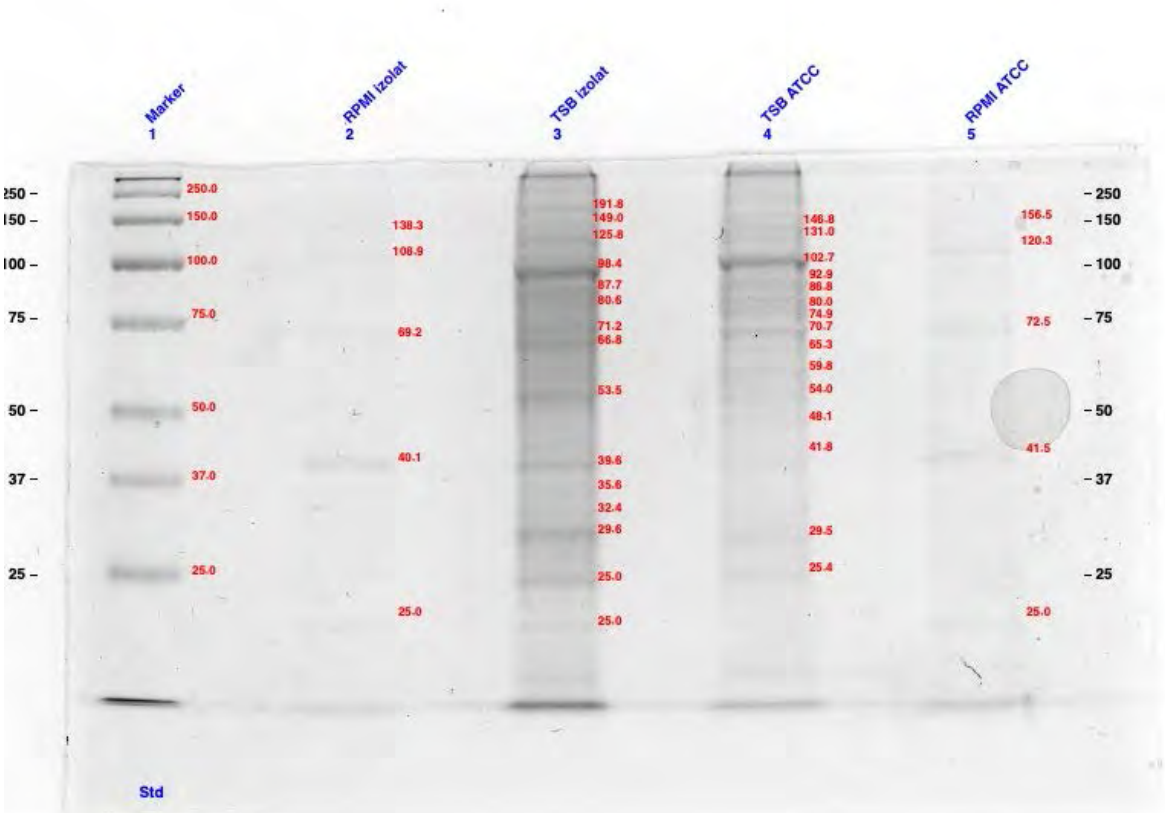
Elektroforez yürütme solüsyonu [Running Buffer; 1.92 M Glycine 14.4 gr, 0.25 M Tris Base 3 gr, %1 SDS 1 gr, 1000 ml saf su (dH_2O)] hazırlanıp jel tankına (Mini-PROTEAN Tetra Cell) eklendi. Örnek proteinler, yükleme jelinde 40V sabit akımda 30 dakika, yürütme jeline geçildiğinde ise 120 Volt sabit akımda yaklaşık 2 saatte ayrıştırıldı. Yürütme tamamlandıktan sonra jel boyama çözeltisinin içine konularak (Coomassie Blue Staining, Thermo) yatay çalkalayıcıda (MR-12, BIOSAN) 3-4 saat bekletildi.

Boyama solüsyonundan alınan jel, boyayı uzaklaştırmak için hazırlanan destaining buffer [(200 ml Methanol, 100 ml Glacial asetik asit, 700 ml saf su (dH_2O)] içerisine konuldu. Jel boyandıktan sonra büyüklüklerine göre ayrışmış proteinler gözlendi.

Bulgular

İdentifikasyon için PZR yapılan *C. pseudotuberculosis* numuneleri 203 bp'lik DNA bantları gözlendiğinden pozitif olarak kabul edildi (Sakmanoğlu ve ark., 2015).

Bu çalışmada SDS-PAGE analizi yapılan 2 adet suş ve 2 adet farklı besiyerinin farklı protein profilleri gösterdiği belirlenmiştir (Şekil 1). RPMI'de üretilen saha izolatın proteinleri sırasıyla; 25 kDa, 40.1 kDa, 59.2 kDa, 108.9 kDa ve 138.3 kDa olarak görülürken, RPMI ATCC suşunda sırasıyla; 25 kDa, 41.5 kDa, 72.5 kDa, 120.3 kDa ve 156.5 kDa luk bantlar saptandı. TSB de üretilen saha suşunda sırasıyla; 25 kDa, 29.6 kDa, 32.4 kDa, 35.6 kDa, 39.6 kDa, 53.5 kDa, 66.8 kDa, 71.2 kDa, 80.6 kDa, 87.7 kDa, 98.4 kDa, 125.8 kDa, 149 kDa, 191.8 kDa bantları görüldü. TSB'de üretilen ATCC suşunda sırasıyla; 25.4 kDa, 29.5 kDa, 41.8 kDa, 48.1 kDa, 54 kDa, 59.8 kDa, 65.3 kDa, 70.7 kDa, 74.9 kDa, 80 kDa, 86.8 kDa, 92.9 kDa, 102.7 kDa, 131 kDa, 145.8 kDa luk bantları görüldü.



Şekil 1. *Corynebacterium pseudotuberculosis* suşlarının SDS-PAGE ile analizi.
1: Marker, 2: *C. pseudotuberculosis* saha izolatı RPMI kültürü, 3: *C. pseudotuberculosis* saha izolatı TSB kültürü, 4: *C. pseudotuberculosis* ATCC 19410 izolatı TSB kültürü, 5: *C. pseudotuberculosis* ATCC 19410 izolatı RPMI kültürü.

RPMI'de üretilen *C. pseudotuberculosis* saha izolatı ile ATCC 19410 izolatının protein profilleri değerlendirildiğinde, her iki izolatta da 5'er protein bandı bulunduğu, 25 kDa büyüklüğündeki proteinin ortak olduğu ve diğer 4 proteinin farklı kDa'lara sahip olduğu görülmektedir.

TSB'de üretilen *C. pseudotuberculosis* saha izolatı ile ATCC 19410 izolatının protein profilleri değerlendirildiğinde, saha izolatında 25 kDa-191.8 kDa arasında değişen 14 bant bulunurken ATCC 19410 izolatında 25.4 kDa-145.8 kDa arasında değişen 15 bant bulunmakta ve benzer proteinlere sahip olmadıkları görülmektedir.

Tartışma ve Sonuç

Kazeöz lenfadenit, dünya genelinde endemik olarak ortaya çıkan ve küçük ruminantlarda çok büyük maddi kayıplara sebep olan bir hastalıktır. OIE'nin 2009 yılındaki yayınlamış olduğu verilerde 1996-2004 yıllarını kapsayan süreçte 201 tane ülkeden alınan veriler karşılaştırılmış olup, 64 tane ülkede hastalığın görüldüğü ve bu ülkelerin de 19 tanesinin Amerika Kıtasında, 18 tanesinin Afrika Kıtasında, 14 tanesinin Avrupa Kıtasında, 11 tanesinin Asya Kıtasında ve 2 tanesinin de Okyanusya'da görüldüğü bildirilmiştir (OIE 2009). Ülkelere göre enfeksiyonun prevalansıyla ilgili oldukça farklı değerler gözlenmiştir. Bu oran, yaklaşık olarak %8-90 aralığında değişkenlik göstermektedir (Ali ve ark., 2016; Kuria ve Holstad, 1989; Middleton ve ark., 1991; Stanford ve ark., 1998). Kazeöz lenfadenitin prevalansıyla ilgili değerlendirmeler, genellikle enfeksiyonun görüldüğü eksternal formdaki lezyonlara bakılarak yapılmaktadır. Bununla beraber hastalığın gerçek prevalansının saptanmasında internal formdaki lezyonlara da özellikle önem verilmesi gerekmektedir (Abebe ve Tessema, 2015).

Açlık stresi, bakterinin protein ekspresyonunu azaltarak enerji tasarrufu yapmasına yardımcı olur. Bu, bakterinin stres faktörlerine karşı direncini artırmasına, bakterinin hücre duvarını güçlendirmesine, hücre içi stresi azaltmaya yardımcı olabilir. Açlık stresinin, ayrıca yeni proteinlerin ekspresyonunu tetikleyerek bakterinin strese karşı adaptasyonuna, bakterinin stres faktörlerinden kaçmasına veya konakçı hücrelerden kurtulmasına katkı sağlayabileceği bildirilmiştir (Lee, J.H., 2017).

Çalışmamızda RPMI besiyerinde üreyen suşlar büyüklükleri 25-157 kDa arasında değişen yaklaşık 5 band sergilerken TSB'de üreyen suşlar büyüklükleri 25-192 kDa arasında değişen yaklaşık 15 band sergilemişlerdir. Bakterinin çok sayıda virulens faktörü bulunmaktadır. Bunlar başta fosfolipaz D olmak üzere farklı moleküler ağırlıklara sahip ATP bağlayıcı sitoplazmik membran proteinleri (30-60 kDa), demir bağlayıcı siderefor proteinleri (Ferritin, Siderophores, 50-100 kDa), fagositozdan kaçınma proteinleri (LcrV, LcrM, LcrP, 10-30 kDa), bakteriyel duvar proteinleri (M protein, 20-100 kDa, bakteriyel zar proteinleri (Fimbriae, Porins, 10-50 kDa), bakteriyel enzim proteinleri (Katalaz, Superoksit dismutaz, DNA polimeraz, 10-100 kDa) arasında molekül ağırlıklarına sahiptirler (Egerton ve ark.,1997).

Bu çalışmada, RPMI besiyerinde açlık stresine maruz kalan suşların SDS-PAGE analizinde ortaya koyduğu bant sayısı, genel besiyerinde üreyen suşların üçte biri kadar olmuştur. Bunun muhtemel nedeni bol miktarda besine sahip oldukları için genel besiyerlerinde, bakterinin daha hızlı üremesi ve bakteriyel enzim protein düzeylerinin artması olabilirken diğer bir nedeni de açlık stresi altında enerji tasarrufu nedeniyle sadece hayatta kalma ile ilgili gen ürünlerinin ifadesine bağlı olması kuvvetle muhtemeldir. Açlık stresine maruz kalan suşların sergilediği proteinlerin büyük bir olasılıkla 50-100 kDa luk siderefor proteinleri olduğu düşünülmektedir. Açlık stresine maruz kalan bakterilerin hayatta kalmaları için zorunlu olan demiri elde etmek üzere bu proteinlerin ifadelerini artırması, bu strese karşı bir adaptasyon mekanizması olarak değerlendirilmektedir (Lee ve ark., 2017). Bu farklılıklar, açlık stresinin bakterinin protein üretimini nasıl etkilediğini göstermektedir. Açlık stresi altında, bakteri enerji tasarrufu sağlamak için ATP üretimini artırmaya, demir alımını artırmaya ve fagositozdan kaçmaya odaklanır. Bu nedenle, bu işlevlerle ilişkili proteinlerin düzeylerinde artış görülür (Lee ve ark., 2017).

Açlık stresine maruz kalan *C. pseudotuberculosis* izolatı ile SDS-PAGE analizi kullanılarak yapılan bir çalışmada, açlık stresinin bakterinin toplam protein ekspresyonunu %30-50 oranında azaltmış olup, bakterinin enerji tasarrufu yapmasına yardımcı olduğu belirtilmiştir (Dorella ve ark., 2006)

Sonuç olarak, açlık stresine ve genel besiyerlerine maruz kalan *C. pseudotuberculosis* suşlarında görülen protein bantları, bakterinin hayatta kalmasını ve büyümesini sağlamaya yardımcı olan çeşitli işlevlerle ilişkilidir. Bu çalışmada, strese maruz bırakılan *C. pseudotuberculosis* suşları arasında protein yapıları arasında profil farklılıkları olduğu tespit edilmiştir. Bu durum bakterinin enerji tasarrufu sağlamak için gereksiz proteinlerin ekspresyonunu azaltması olabilir. RPMI da üreyen suşların sergilediği, ancak TSB'de üreyen suşların sergilemediği proteinlerin büyük bir olasılıkla 50-100 kDa luk siderefor proteinleri olduğu düşünülmektedir. *C. pseudotuberculosis* suşlarının açlık stresine maruz bırakıldığında demir metabolizması ile ilgili protein düzeylerindeki değişikliklerin, daha net bir şekilde ortaya konması için, açlığa maruz kalan ve kalmayan *C.pseudotuberculosis* suşlarının tüm hücre proteinlerinin antijen olarak kullanıldığı ve açlık stresinde arttığı düşünülen siderefor virülans faktörlerine karşı monoklonal antikorlar kullanılarak yapılacak immunoblot testi çalışmalarına ihtiyaç duyulmaktadır.

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Fikir/Kavram: SEG
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Denetleme/Danışmanlık: SEG
Veri Toplama ve/veya İşleme: MD, SEG
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Kaynak Taraması: MD, SEG
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Kaynaklar

Abebe D, Tessema TS. 2015: Determination of *Corynebacterium pseudotuberculosis* prevalence and antimicrobial susceptibility pattern of isolates from lymph nodes of sheep and goats at an organic export abattoir, Modjo, Ethiopia. *Lett Appl Microbiol*. 61, 469-476.

Ali AD, Mahmoud AA, Khadr AM, Elshemey TM, Abdelrahman AH. 2016: *Corynebacterium pseudotuberculosis*: disease prevalence, lesion distribution, and diagnostic comparison through microbiological culture and molecular diagnosis. AJVS. 51(2): 189-198.

Aslı Sakmanoğlu, Hasan Hüseyin Hadimli, Osman Erganiş, Yasemin Pınarkara, Zafer Sayın, Kürşat Kav. 2015: Koyunlardan izole edilen *Corynebacterium pseudotuberculosis* suşlarının identifikasyonu ve antibiyotiklere duyarlılıkları. Eurasian J Vet Sci. 31, 2, 116-121. DOI:10.15312/EurasianJVetSci.2015210083.

Bastos BL, Portela RWD, Dorella FA, Ribeiro D, Seyffert N, Castro TLP, Miyoshi A, Oliveira SO, Meyer R, Azevedo V. 2012: *Corynebacterium pseudotuberculosis:* immunological responses in animal models and zoonotic potential. *J Clin Cell Immunol*. S4:005: doi:10.4172/2155-9899.S4-005.

Billington SJ, Esmay PA, Songer JG, Jost BH. 2002: Identification and role in virulence of putative iron acquisition genes from *Corynebacterium pseudotuberculosis*. *FEMS Microbiology Letters.* 208:41–45 DOI 10.1111/j.1574-6968.2002.tb11058.x.

Dorella FA, Pacheco LGC, Olivera SC, Miyashi A, Azevedo V. 2006: *Corynebacterium pseudotuberculosis:* microbiology, biochemical properties, pathogenesis and molecular studies of virulence. *Vet Res*, 37: 201-218.

Egerton D.J., Buxton P.A. 1997: Characterization of detergent-soluble proteins of *Corynebacterium pseudotuberculosis*. Journal of Applied Microbiolog. 82(6), 673-680.

Gomide ACP, Ibraim IC, Alves JTC, de Sá PG, de Oliveira Silva YR, Santana MP, Silva WM, Folador EL, Mariano DCB, de Paula Castro TL, Barbosa S, Dorella FA, Carvalho AF, Pereira FL, Leal CAG, Figueiredo HCP, Azevedo V, Silva A, Folador ARC. 2018: Transcriptome analysis of *Corynebacterium pseudotuberculosis* biovar equi in two conditions of the environmental stress. Gene. 677:349-360. doi: 10.1016/j.gene.2018.08.028. Epub 2018 Aug 8. PMID: 30098432.

Güllü Yücetepe A., 2023: Şanlıurfa Bölgesindeki Evcil Kanatlılardan *Mycoplasma gallisepticum* İzolasyonu ve Moleküler Karakterizasyonu. Doktora Tezi, Harran Üniversitesi, Şanlıurfa.

İlhan Z. 2003: Koyunlarda *Corynebacterium pseudotuberculosis*'in ELISA ve dot-blot ELISA ile teşhisi. *Turk J Vet Anim Sci*. 27(6): 1327-1333.

Kuria JK, Holstad G. A. 1989: Seroepidemiological investigation of *Corynebacterium pseudotuberculosis* infection in sheep flocks in southern Norway. Acta Vet Scand. 30: 107–108.

Laemmli UK. 1970: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227(5259):680-5.

Lee, J. H., Kim, J. Y., Lee, Y. J. 2017: Effect of starvation on the protein expression profile of *Corynebacterium pseudotuberculosis*. Antonie van Leeuwenhoek, 110 (12), 2565-2575.

Middleton MJ, Epstein WM, Gregory GG. 1991: Caseous lymphadenitis on Flinders Island: prevalence and management surveys. *Aust Vet J.* 68: 311–312.

OIE World Health Organisation for Animal Health. Available at: http://www.oie.int/hs2/sit_mald_cont.aspc_mald=156andc_cont=6andannee=2004. 2009 (Accession date: 02.02.2020).

Öztürk, A., Karaca, M. 2023: *Brucella* spp. Efficacy of agarose gel electrophoresis method for isolation and identification. Turkish Journal of Clinical Microbiology and Infectious Diseases. 35(3), 279-283.

Pacheco LGC, Pena RR, Castro TLP, et al., 2007: Multiplex PCR assay for identification of *Corynebacterium pseudotuberculosis* from pure cultures and for rapid detection of this pathogen in clinical samples. J Med Microbiol. 56, 480- 486.

Parin U, Kırkan Ş, Ural K, Savaşan S, Erbaş G, Gültekin M, Yüksel HT, Balıkçı C. 2018: Molecular identification of *Corynebacterium pseudotuberculosis* in sheep. Acta Vet Brno. 87: 3-8.

Ribeiro D, Rocha Fde S, Leite KM, Soares Sde C, Silva A, Portela RW, Meyer R, Miyoshi A, Oliveira SC, Azevedo V, Dorella FA. 2014: An iron-acquisition-deficient mutant of *Corynebacterium pseudotuberculosis* efficiently protects mice against challenge. Veterinary Research 45(1):28 DOI 10.1186/1297-9716-45-28.

Ruiz JC, D'Afonseca V, Silva A, Ali A, Pinto AC. 2011: Evidence for reductive genome evolution and lateral acquisition of virulence functions in two *Corynebacterium pseudotuberculosis* strains. PLoS One. 6(4):e18551.

Stanford K, Brogden KA, McClelland LA, Kozub GC, Audibert F. 1998: The incidence of caseous lymphadenitis in Alberta sheep and assessment of impact by vaccination with commercial and experimental vaccines. Can J Vet Res. 62: 38-43.

HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Isolation and Characterization of Cefotaxime and Ciprofloxacin Co-Resistant *Escherichia coli* in Retail Chicken Carcasses

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Abstract: Transmission of antimicrobial-resistant bacteria to humans through the food chain is of great importance for public health. In this study, it was aimed to isolate and characterize the cefotaxime and ciprofloxacin-resistant *Escherichia coli* in retail chicken meat samples sold in Hatay. The isolates were subjected to phylogenetic group typing and antimicrobial susceptibility testing. The genetic relatedness of the isolates was determined using Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) technique. The isolates were also screened for the presence of both antimicrobial and plasmid-mediated quinolone resistance (PMQR) genes by PCR. Cefotaxime and ciprofloxacin co-resistant *E. coli* isolates with diverse genetic origins were recovered in 42.3% (22/52) of retail chicken carcasses. The *E. coli* isolates belonged to the phylogenetic group D2 were dominant (40.9%, 9/22), followed by B1 (27.3%, 6/22), $B2_3$ (18.2%, 4/22), and A1 (13.6%, 3/22), respectively. Based on dendrogram analysis, the ERIC-PCR method differentiated the isolates into 10 clusters (I-X). The multidrug resistance (MDR) was observed in 81.8% (18/22) of the isolates. PMQR determinants were not identified in any isolates tested. Molecular analysis revealed one or more β-lactamase-encoding genes in all isolates as a single or in combination: bla_{CTX-M}-bla_{TEM} (n=5), bla_{CMY-2} (n=5), bla_{CTX-M} (n=5), bla_{CMY-2}-bla_{SHV} (n=3), bla_{CMY-2}-bla_{TEM} (n=3), and bla_{CTX-M}-bla_{CMY-2} (n=1). This study highlights that retail chicken meat is an important reservoir of cefotaxime and ciprofloxacin co-resistant *E. coli* isolates. It is necessary to evaluate their contribution to the community and hospital infections.

Keywords: *Antimicrobial resistance, Chicken carcasses, ERIC-PCR, Escherichia coli, Phylogenetic typing.*

Perakende Tavuk Karkaslarından Sefotaksim ve Siprofloksasin Eş Dirençli *Escherichia coli* İzolasyonu ve Karakterizasyonu

Özet: Antimikrobiyal dirençli bakterilerin gıda zinciri yoluyla insanlara bulaşması halk sağlığı açısından büyük önem taşımaktadır. Bu çalışmada Hatay'da satışa sunulan perakende tavuk eti örneklerinde sefotaksim ve siprofloksasine dirençli *Escherichia coli*'nin izolasyonu ve karakterizasyonu amaçlandı. İzolatlar filogenetik grup tiplendirmesine ve antimikrobiyal duyarlılık testlerine tabi tutuldu. Ayrıca izolatlar arasındaki genetik yakınlığı belirlemek için Enterobacterial Repetitive Intergenic Consensus Polimeraz Zincir Reaksiyonu (ERIC-PZR) tekniği kullanıldı. Plazmit aracılı kinolon direnci (PMQR) ile diğer direnç genleri PCR ile araştırıldı. Perakende tavuk karkaslarının %42.3'ünden (22/52) farklı genotipe sahip sefotaksim ve siprofloksasine dirençli *E. coli* izole edildi. İzolatlar arasında dominant filogenetik grup D2 (%40.9, 9/22) olup; bunu sırasıyla B1 (%27.3, 6/22), $B2_3$ (%18.2, 4/22) ve A1 (%13.6, 3/22) filogrupları izledi. Dendrogram analizine dayalı olarak, ERIC-PCR yöntemi izolatları 10 kümeye (I-X) ayırdı. İzolatların %81.8'inde (18/22) çoklu ilaç direnci (MDR) belirlendi. PMQR genleri izolatların hiçbirinde tespit edilmezken, diğer sınıftan antimikrobiyallere dirence aracılık eden çok sayıda gen saptandı. İzolatlarda β-laktamaz sentezinden sorumlu genlerin tek veya kombine olarak bulunduğu görüldü: bla_{CTX-M}-bla_{TEM} (n=5), bla_{CMY-2} (n=5), bla_{CTX-M} (n=5), bla_{CMY-2}-bla_{SHV} (n=3), bla_{CMY-2}-bla_{TEM} (n=3), and bla_{CTX-M}-bla_{CMY-2} (n=1). Bu çalışma, perakende tavuk etinin, sefotaksim ve siprofloksasine dirençli *E. coli* izolatları için önemli bir rezervuar olduğunu göstermiştir. Toplum ve hastane enfeksiyonlarına katkılarının değerlendirilmesi gerekmektedir.

Anahtar Kelimeler: *Antimikrobiyal direnç, ERIC-PCR, Escherichia coli, Filogenetik tiplendirme, Tavuk karkas.*

Introduction

The misuse and overuse of antibiotics in food animals for different purposes (treatment, prophylaxis, feed additive, etc.) have led to the selection and spread of antibiotic-resistant *Escherichia coli* strains (Ramos et al., 2020**)**. Poultry meat production and consumption have increased significantly worldwide and are expected to increase in the coming decades (Klaharn et al., 2022). Poultry meat is considered a potential vehicle for foodborne pathogens and resistant bacteria, making it a major public health problem worldwide (Gonçalves-Tenório et al., 2018). On the other hand, this situation also has a high global impact on human health and socioeconomic burden (Buzby et al., 2009; Parisi et al., 2020; WHO, 2015). Bacterial contamination has been shown to occur at every stage of the production chain from farm to table (Ananchaipattana et al., 2012; Heyndrickx et al., 2002). However, contamination mostly occurs during slaughtering processes; plucking, evisceration, and chilling have been shown to be the most important operations. It is therefore strongly suggested that an improvement in hygiene practices throughout the food chain is required to reduce the risk of foodborne pathogens and resistant bacteria from poultry meat products (Klaharn et al., 2022). Resistant *E. coli* isolates can cause intestinal and extra-intestinal infections as a result of the consumption of contaminated chicken meat and ready-to-eat chicken meat products (Davis et al., 2018).

Ciprofloxacin, a quinolone class antibiotic, inhibits DNA replication, while cefotaxime, a third-generation cephalosporin antibiotic, prevents bacterial cell wall synthesis, thereby hindering bacterial proliferation. Both antibiotics play a crucial role in clinical practice, used to combat various bacterial infections. However, excessive and inappropriate usage can lead to the development of resistance, limiting treatment options and posing a serious public health concern. This study aimed to search for the presence of ciprofloxacin and cefotaxime co-resistant *E. coli* isolates in retail chicken carcasses and to perform their molecular characterization.

Material and Methods

Ethic Statement

This study is not subject to HADYEK permission in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees".

Chicken Carcasses

From March to July 2023, retailed chicken carcasses were purchased from different markets in Hatay and its districts. The chicken carcasses were transported on ice within six hours to the laboratory for examination.

Isolation of *E. coli* resistant to cefotaxime and ciprofloxacin

Chicken carcasses were thoroughly rinsed with 500 ml buffered peptone water (BPW) and incubated for 18-20 hours at 37 °C. Following the incubation period, 100 µl of the culture was taken and transferred to 10 ml EC broth and incubated for 20-22 hours at 44 °C. A loopful of this culture was taken and plated on MacConkey medium containing 4 µg/ml ciprofloxacin and 8 µg/ml cefotaxime and incubated for 22–24 hours at 37 °C (CLSI, 2022). One of the typical brick red-coloured colonies were selected and identified with classical biochemical tests. Isolates identified as *E. coli* were stored at -80 °C in LB broth containing 20% glycerol until antimicrobial susceptibility testing and molecular analysis are performed.

Antimicrobial susceptibility testing

Antimicrobial susceptibilities of *E. coli* isolates were performed and evaluated in line with the Clinical Laboratory Standards Institute (CLSI) using disk diffusion method (CLSI, 2022). The following antibiotic disks were used for the determination of susceptibilities of the isolates: ampicillin (AM, 10 µg), cefoxitin (FOX, 30 µg), amoxicillin-clavulanic acid (AMC, 10/20 µg), cefotaxime (CAZ, 30 µg), cefepime (FEB, 30 µg), meropenem (MEM, 10 µg), gentamicin (CN, 10 µg), tobramycin (TOB, 10 µg), amikacin (AK, 10 µg), tetracycline (TE, 30 µg), sulfamethoxazole-trimethoprim (SXT, 1.25/23.75 µg), ciprofloxacin (CIP, 5 µg) and chloramphenicole (C, 30 µg). *E. coli* ATCC 25922 was used as a control strain for antimicrobial susceptibility testing.

Determination of MIC values of CIP^R-CTX^R *E. coli* isolates

MIC values of ciprofloxacin (0.002-32 µg/ml) and cefotaxime (0.016-256 µg/ml) resistant isolates for these antimicrobials were determined by E-test.

Phylogenetic typing

Phylogenetic types of CIP^R-CTX^R *E. coli* isolates were determined using primers targeting *chuA*, *yjaA* and *TSPE4.C2* genes by Clermont et al. (2000). Determination of phylogenetic groups was done based on the profiles of these three genes (*chuA*/*yjaA*/*TSPE4.C2*): A0 (−/−/−), A1 (−/+/−), B1 (−/−/+), $B2_2$ (+/+/−), $B2_3$ (+/+/+), D1 (+/−/−) and D2 (+/−/+) by Escobar-Páramo et al. (2004).

Investigation of resistance genes in *E. coli* isolates

Resistance genes mediating resistance to aminoglycoside *(aac(3)-IV, aadA, strA/B, aadB, aphA1,* and *aphA2),* trimethoprim-sulfamethoxazole *(sul1, sul2, sul3, dhfrI, dhfrIII, dhfrV, dhfrIX, and dhfrXIII),* chloramphenicol *(catI, catII,* and *catIII),* cefotaxime *(bla_{CTX-M}, bla_{SHV}, bla_{TEM}),* tetracycline *(tetA, tetB, tetC, tetD, tetE,* and *tetG),* amoxicillin-clavulanic acid and cefoxitin *(bla_{CMY-2})* were searched by PCR as previously reported (Kozak et al. 2009; Monstein et al. 2007; Ng et al. 2001; Zhao et al. 2001). Screening of PMQR genes in ciprofloxacin-resistant isolates (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac(6')-Ib* and *qepA* genes) were investigated as per Cavaco et al. (2009), Kim et al. (2009), and Park et al. (2006).

Genotyping of isolates by Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR)

Molecular genotyping of *E. coli* isolates was performed by ERIC-PCR using specific primers (Versalovic et al., 1991). The bands for each isolate were counted using the zero-one manual method, the data was then entered into the

following site: http://insilico.ehu.es/dice_upgma/, dendrograms were plotted.

Results

Of the 52 tested chicken carcass samples, 22 (42.3%) ciprofloxacin and cefotaxime co-resistant *E. coli* isolates were recovered. Based on the CLSI clinical cut-off values for CIP^R (cut-off ≥4 µg/ml) and CTX^R (cut-off ≥16/ml) in *Enterobacterales*, and all isolates (n = 22) exhibited MICs above the threshold values. All CIP^R-CTX^R *E. coli* isolates were resistant to AMP, but susceptible to ME, FEB and AK. The highest resistance rate was detected against SXT (72.7%, 16/22), followed by TE (59.1%, 13/22), C (59.1%, 13/22), AMC (54.5%, 12/22), FOX (45.5%, 10/22), CN (36.4%, 8/22), and TOB (18.2%, 4/22). Regarding the ERIC-PCR profiles, the isolates were differentiated into ten clusters (I-X). ERIC-PCR profiles of representative E. coli isolates were given in Figure 1.

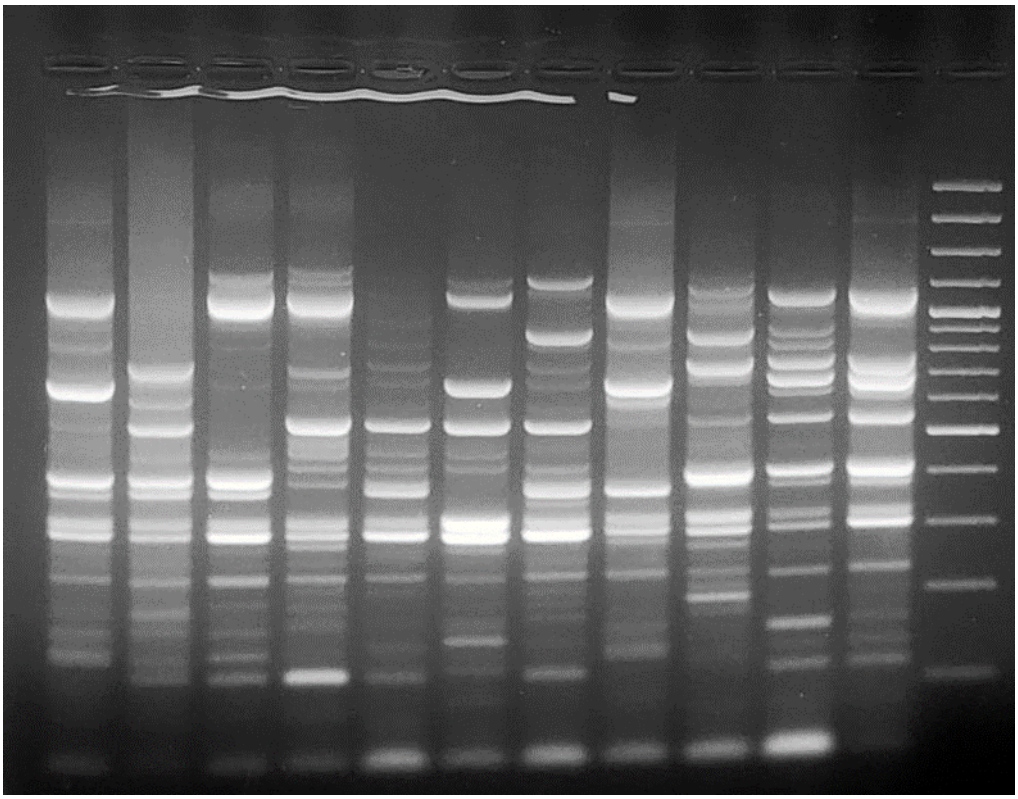

Figure 1. ERIC-PCR profiles of representative *E. coli* isolates.

PMQR genes were not among the isolates. All CIP^R-CTX^R isolates carried at least one of the beta-lactamase genes. The most predominant beta-lactamase gene was observed as bla_{CMY-2}, which was detected in 12 (54.5%) isolates. Among the beta-lactamase genes examined, bla_{CTX} in 11 (50%) isolates, bla_{TEM} in 8 (36.4%) isolates, and bla_{SHV} in 3 (13.6%) isolates was detected. The tetracycline resistance was associated with *tetA* and *tetB* genes, of which *tetA* was present in 12 isolates, and *tetB* in seven isolates. Among gentamicin-resistant isolates (n=8), *aac(3)-IV* (n=5) and *aadB* (n =3) were the only genes detected. In addition, several aminoglycoside resistance genes including *aadA*, *strA/B*, *aph1*, and *aph2* were detected in these isolates as well. The *catI* (n=6) and *catII* (n=4) genes were only genes associated with chloramphenicol-resistance, however, none of the isolates carried *catIII*. The *sul1*, *sul2*, and *sul3* genes encoding sulfonamide resistance were found in nine, 12, and five *E. coli* isolates, respectively. Additionally, the genes responsible for trimethoprim resistance [*dhfrI* (n=9), *dhfrV* (n=3), *dhfrIX* (n=3) and *dhfrXIII* (n=5)] were also detected in SXT-resistant isolates (Figure 2).

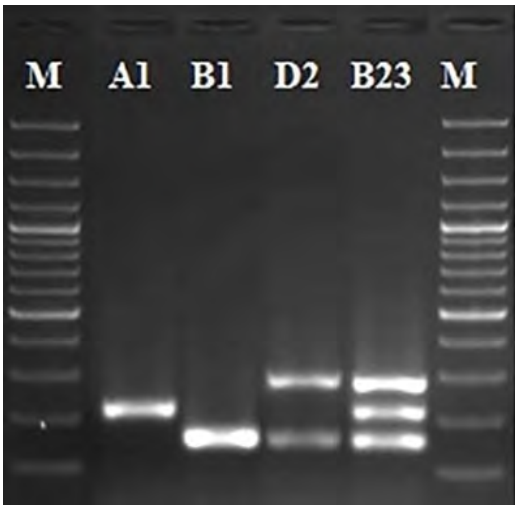

Figure 3. Phylogenetic groups determined among *E. coli* isolates

The phylogenetic analysis showed that the predominant phylogroup was D2 (n=9), followed by B1 (n=6), $B2_3$ (n=4), and A1 (n=3) in *E. coli* isolates (Figure 3).

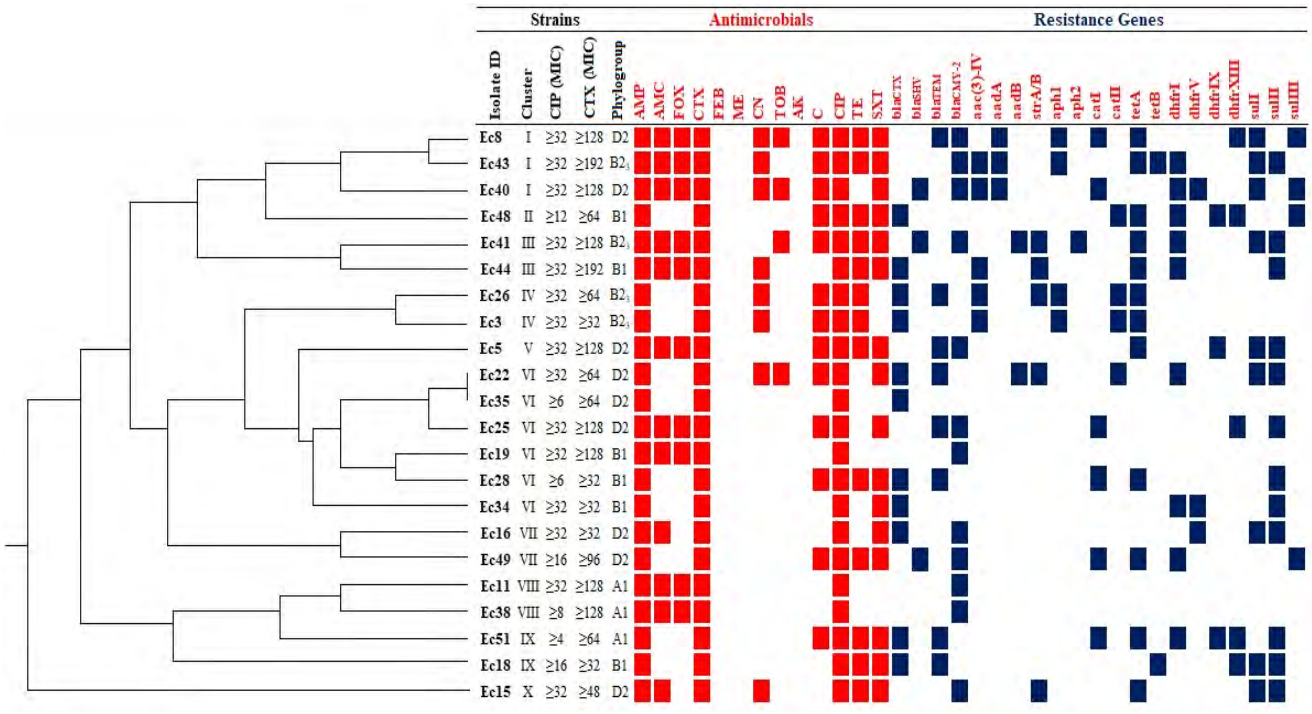

Figure 2. The dendrogram constructed based on ERIC-PCR profiles.
The antimicrobial resistance and its related genes are indicated with red and blue squares, respectively. AMP; ampicillin, AMC; amoxycillin-clavulanic acid, FOX; cefoxitin, CTX; cefotaxime, FEB; cefepime, ME; meropenem, AK; amikacin, TOB; tobramycin, CN; gentamicin, C; chloramphenicol, TE; tetracycline, CIP; ciprofloxacin, SXT; trimethoprim-sulfamethaxole.

Discussion

In the current study, ciprofloxacin and cefotaxime co-resistant *E. coli* strains with diverse genetic origins were isolated in 42.3% (22/52) of retail chicken carcasses samples sold in Hatay province. The findings of this study indicated that chicken carcasses were a growing source of MDR bacteria that pose significant public health threats due to the transfer of these resistant bacteria from food-producing animals and their products to the community. This highlighted the increasing problem of CIP^R-CTX^R *E. coli* in chicken carcasses for public health (Xu et al. 2014; Şahin et al. 2020).

The ERIC-PCR profiles of CIP^R-CTX^R *E. coli* isolates demonstrated a high genetic heterogeneity, indicating that contamination of chicken retail meat with these resistant isolates originated from different sources and the high transmission potential of these isolates (Xu et al. 2014). A similar observation was reported by Şahin (2020), who determined high clonal diversity among CIP^R isolates based on pulsed-field gel electrophoresis (PFGE). On the other hand, Wasyl et al. (2014) explained diverse *XbaI*-PFGE profiles in commensal quinolone-resistant *E. coli* isolates by antimicrobial pressure causing the selection of random *gyrA* and *parC* mutants.

In the present study, most of the CTX^R and CIP^R *E. coli* isolates had MDR profiles (81.8%, 18/22). In addition to ciprofloxacin and cefotaxime resistance, higher resistance rates to AMP (100%), SXT (72.7%), TE (63.6%), C (59.1%), AMC (54.5%), FOX (45.5%), and CN (36.4%) were determined, of which some were widely used antimicrobials in food-producing animals. In a study conducted by Şahin (2020), CIP^R *E. coli* isolates were found to show higher resistance rates to AMP (94.9%), SXT (76.3%), and TE (69.5%). Moreover, Xu et al. (2014) in China reported higher resistance rates to SXT (96.3%), TE (92.5%), C (90%), and CN (46.3%) in CIP^R-CAZ^R *E. coli* strains isolated from whole chicken carcasses. This could be explained by the fact that the high capacity of *E. coli* to accumulate resistance genes through horizontal gene transfer (Vanstokstraeten et al., 2023). The fact that CIP^R-CTX^R isolates also develop resistance to other classes of antimicrobials, makes treatment options for infections caused by these factors very limited, and can significantly lead to high mortality and financial burden (WHO, 2021).

In previous studies conducted in the region, *bla*$_{CTX-M}$ was reported as the dominant type in ESBL-producing *E. coli* from poultry meat, and being alone or mostly together with *bla*$_{TEM}$ gene (Kürekçi et al., 2019; Pehlivanlar Önen et al., 2015). In contrast to our study, most of the isolates (54.5%, 12/22) carried *bla*$_{CMY-2}$, which was alone in five isolates and in seven isolates in combination with other beta-lactamase genes. Similarly, Bilge et al. (2020) reported a high prevalence of the plasmid-mediated AmpC beta-lactamase gene, *bla*$_{CMY}$ (65%, 13/20), among cephalosporin-resistant *E. coli* isolates obtained from chicken meats. Besides, Aslantaş (2020) reported a higher prevalence of plasmid-mediated AmpC type beta-lactamase (pAmpC), *bla*$_{CMY-2}$, in broiler flocks in the region, but to a lesser extent *bla*$_{CTX-M}$. This indicates the changing epidemiology of ESBL-producing *E. coli* in broiler flocks.

Point mutations in the *gyrA* and *parC* genes in Gram-negative bacteria, including *E. coli*, are the main mechanism of quinolone resistance. Furthermore, several PMQR

mechanisms have been reported, some of which encode efflux pumps (*QepA*, *OqxAB*), quinolone-modifying enzymes (acetyltransferase *aac(6')-Ib-cr*), and protective proteins (QnrABCDS) (*qnrA*, *qnrB*, *qnrC*, *qnrD*, and *qnrS*) (Imkamp et al., 2023). In this study, the isolates were not examined for mutations on *gyrA* and *parC* genes. However, only PMQR genes were searched in the isolates and no PMQR gene was found in any isolate. Similarly, Şahin (2020) reported that CIP^R *E. coli* strains obtained from chicken meat samples were negative for PMQR genes. Additionally, in another study, a low-level presence of the PMQR genes was reported in ESBL-producing *E. coli* strains from chicken meat samples (Kürekci et al., 2018). The authors reported the presence of only *qnrS* (9.6%) and *qnrB* (15.4%) genes in ESBL-producing *E. coli* strains, but did not detect other PMQR genes (*qnrA*, *qnrC*, *qnrD*, and *aac(6')-Ib-cr*). Therefore, the ciprofloxacin resistance observed in this study could be attributed to point mutations in the quinolone resistance-determining region (QRDR) of the *gyrA* and *parC* genes.

Based on phylogenetic grouping, *E. coli* isolates have been divided into four main groups; virulent strains that cause extraintestinal infections are generally in groups B2 and D, while most of the commensal isolates belong to groups A and B1. In this study, while most of the isolates belonged to the D2 (40.9%, 9/22) and $B2_3$ (18.2%, 4/22) phylogenetic groups, which include virulent strains, the rest of the isolates belonged to B1 (27.3%, 6/22) and A1 (18.2%, 4/22) phylogenetic groups, which includes commensal strains. Soufi et al. (2009) reported a high prevalence (91%) of virulence-associated genes in the *E. coli* isolates belonging to phylogenetic groups B2 and D. In contrast to our study, it has been reported that there are *E. coli* isolates belonging to low virulent phylogroups among those obtained from chicken meat origin. In China, Wu et al. (2015) reported phylogroup B1 (33.5%) in *E. coli* isolates from broiler carcasses and Xu et al. (2014) reported group A (59.4%) in CIP^R-CTX^R *E. coli* isolates from retail broiler carcasses as predominant group. Kürekci et al. (2018) reported the dominance of phylogroup D among ESBL-producing *E. coli*, but the presence of phylogroup B2 only in a few isolates. In Spain, Egea et al. (2012) reported that B1 and A1 accounted for more than 60% of ESBL-producing *E. coli* recovered raw poultry meat (chicken and turkey). Soufi et al. (2009) attributed the high prevalence of virulence-related genes to the fact that these genes are encoded by pathogenicity islands, which favour the spread of pathogenicity determinants in different ecosystems.

In conclusion, the findings of this study indicate that chicken carcasses are a reservoir of cefotaxime and ciprofloxacin co-resistant isolates with several resistance mechanisms against different classes of antimicrobials, and emphasize the necessity for careful regulation of antibiotic usage. The presence of resistant *E. coli* strains that could be transmitted to humans through contaminated chicken meat and products increases the risk of foodborne infections. It was identified that bacterial contamination intensifies, particularly during slaughtering processes, highlighting the crucial need for improving hygiene standards. Since these isolates have different resistant mechanisms and different genotypes, their pathogenicity should be investigated, and their contributions to community and hospital infections should be evaluated.

Availability of Data and Materials

The authors declare that data supporting the study findings are also available from the corresponding author on reasonable request.

Ethical Statement

The study doesn't require ethical approval from Animal Experiments Local Ethics Committee.

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Competing Interests

The authors declared that there is no conflict of interest.

Author Contributions

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Design: ÖA, AMK, MBN
Supervision/Consultation: ÖA, AMK, MBN
Data Collection and/or Processing: ÖA, AMK, MBN
Analysis and/or Interpretation: ÖA, AMK, MBN
Literature Search: ÖA, AMK, MBN
Writing Manuscript: ÖA, AMK, MBN
Critical Review: ÖA, AMK, MBN

References

Ananchaipattana C, Hosotani Y, Kawasaki S, Pongsawat S, Latiful BM, Isobe S, Inatsu Y, 2012: Prevalence of foodborne pathogens in retailed foods in Thailand. *Foodborne Pathog Dis*, 9 (9), 835-840.

Aslantaş Ö, 2020: High occurrence of CMY-2-type beta-lactamase-producing *Escherichia coli* among broiler flocks in Turkey. *Trop Anim Health Prod*, 52 (4), 1681-1689.

Bilge N, Sezer Ç, Vatansever L, Pehlivanlar Önen S, 2020: Occurrence and molecular characterization of cephalosporin resistant *Escherichia coli* isolates from chicken meat. *Kafkas Univ Vet Fak Derg*, 26 (4): 463-468.

Buzby JC, Roberts T, 2009: The economics of enteric infections: human foodborne disease costs. *Gastroenterology*, 136 (6), 1851-1862.

Cavaco LM, Hasman H, Xia S, Aarestrup FM, 2009: *qnrD*, a novel gene conferring transferable quinolone resistance in Salmonella enterica serovar Kentucky and Bovismorbificans strains of human origin. *Antimicrob Agents Chemother,* 53, 603–608.

Clinical and Laboratory Standards Institute (CLSI), 2022: Performance standards for antimicrobial susceptibility testing. CLSI Document: M100-32. Wayne, PA, USA.

Clermont O, Bonacorsi S, Bingen E, 2000: Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol*, 10, 4555-4558.

Davis GS, Waits K, Nordstrom L, Grande H, Weaver B, Papp K, Horwinski J, Koch B, Hungate BA, Liu CM, Price LB, 2018: Antibiotic-resistant *Escherichia coli* from retail poultry meat with different antibiotic use claims. *BMC Microbiol*, 18(1),174.

Escobar-Paramo P, Clermont O, Blanc-Potard AB, Bui H, Le Bouguenec C, Denamur E, 2004: A specific genetic background is required for acquisition and expression of virulence factors in *Escherichia coli*. *Mol Biol Evol*, 21, 1085-1094.

Gonçalves-Tenório A, Silva BN, Rodrigues V, Cadavez V, Gonzales-Barron U, 2018: Prevalence of pathogens in poultry meat: a meta-analysis of European published surveys. *Foods*, 7 (5), 69.

Heyndrickx M, Vandekerchove D, Herman L, Rollier I, Grijspeerdt K, De Zutter L, 2002: Routes for Salmonella contamination of poultry meat: epidemiological study from hatchery to slaughterhouse. *Epidemiol Infect*, 129 (2), 253-265.

Imkamp F, Bodendoerfer E, Mancini S, 2023: QUIRMIA-A Phenotype-Based Algorithm for the Inference of Quinolone Resistance Mechanisms in *Escherichia coli*. *Antibiotics (Basel)*, 12 (7), 1119.

Kim HB, Park CH, Kim CJ, Kim EC, Jacoby GA, Hooper DC, 2009: Prevalence of plasmid-mediated quinolone resistance determinants over a 9-year period. *Antimicrob Agents Chemother*, 53, 639–645.

Klaharn K, Pichpol D, Meeyam T, Harintharanon T, Lohaanukul P, Punyapornwithaya V, 2022: Bacterial contamination of chicken meat in slaughterhouses and the associated risk factors: A nationwide study in Thailand. PLoS ONE 17(6): e0269416.

Kozak GK, Pearl DL, Parkman J, Reid-Smith RJ, Deckert A, Boerlin P, 2009: Distribution of sulfonamide resistance genes in *Escherichia coli* and Salmonella from swine and chickens at abattoirs in Ontario and Quebec, Canada. *Appl Environ Microbiol*, 75, 5999-6001.

Kürekci C, Osek C, Aydın M, Tekeli İO, Kurpas M, Wieczorek K, Sakin F, 2019: Evaluation of bulk tank raw milk and raw chicken meat samples as source of ESBL producing *Escherichia coli* in Turkey: Recent insights. *J Food Saf*, 39, (2), e12605.

Monstein HJ, Ostholm-Balkhed A, Nilsson MV, Nilsson M, Dornbusch K, Nilsson LE, 2007: Multiplex PCR amplification assay for the detection of blaSHV, blaTEM and blaCTX-M genes in Enterobacteriaceae. *APMIS*, 115 (12), 1400-1408.

Ng LK, Martin I, Alfa M, Mulvey M, 2001: Multiplex PCR for the detection of tetracycline-resistant genes. *Mol Cell Probes*, 15, 209-215.

Parisi A, Stanaway J, Sarkar K, Crump J, 2020: The global burden of non-typhoidal Salmonella invasive disease: a systematic analysis for the global burden of disease study 2017. *Int J Infect Dis*, 101, 341-359.

Park CH, Robicsek A, Jacoby GA, Sahm D, Hooper DC, 2006: Prevalence in the United States of *aac(6')-Ib-cr* encoding a ciprofloxacin-modifying enzyme. *Antimicrob Agents Chemother,* 50, 3953–3955.

Ramos S, Silva V, Dapkevicius MLE, Caniça M, Tejedor-Junco MT, Igrejas G, Poeta P, 2020: *Escherichia coli* as commensal and pathogenic bacteria among food-producing animals: Health implications of extended spectrum β-lactamase (ESBL) production. *Animals (Basel)*, 10, (12), 2239.

Şahin S, 2020: Determination of the ciprofloxacin-resistant *Escherichia coli* isolated from chicken meat in Turkey. *J Hellenic Vet Med Soc*, 71 (3), 2291–2300.

Pehlivanlar Önen S, AslantaŞ Ö, Yılmaz EŞ, Kürekci C, 2015: Prevalence of β-lactamase producing *Escherichia coli* from retail meat in Turkey. *J Food Sci*, 80, M2023–M2029.

Soufi L, Abbassi MS, Saenz Y, Vinue L, Somalo S, Zarazaga M, Abbas A, Dbaya R, Khanfir L, Ben Hassen A, Hammami S, Torres C, 2009: Prevalence and diversity of integrons and associated resistance genes in *Escherichia coli* isolates from poultry meat in Tunisia. *Foodborne Pathog Dis*, 6, 1067–1073.

Versalovic J, Koeuth T, Lupski R, 1991: Distribution of repetitive DNA sequences in eubacteria and application to fingerpriting of bacterial genomes. *Nucleic Acids Res*, 19 (24), 6823-6831.

Vanstokstraeten R, Piérard D, Crombé F, De Geyter D, Wybo I, Muyldermans A, Seyler L, Caljon B, Janssen T, Demuyser T, 2023: Genotypic resistance determined by whole genome sequencing versus phenotypic resistance in 234 *Escherichia coli* isolates. *Sci Rep*, 13 (1), 449.

WHO, 2021: Antimicrobial resistance. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

WHO, 2015: WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015. https://www.who.int/publications-detail-redirect/9789241565165

Wu H, Xia S, Bu F, Qi J, Liu Y, Xu H, 2015: Identification of integrons and phylogenetic groups of drug-resistant *Escherichia coli* from broiler carcasses in China. *Int J Food Microbiol*, 211, 51-65.

Wasyl D, 2014: Prevalence and characterization of quinolone resistance mechanisms in commensal *Escherichia coli* isolated from slaughter animals in Poland, 2009-2012. *Microb Drug Resist*, 20, 544-549.

Xu X, Cui S, Zhang F, Luo Y, Gu Y, Yang B, Li F, Chen Q, Zhou G, Wang Y, Pang L, Lin L, 2014: Prevalence and characterization of cefotaxime and ciprofloxacin co-resistant *Escherichia coli* isolates in retail chicken carcasses and ground pork, China. *Microb Drug Resist*, 20 (1), 73-81.

Zhao S, White DG, Mc Dermott PF, Friedman S, English L, Ayers S, Meng J, Maurer J, Holland R, Walker RD, 2001: Identification and expression of cephamycinase bla_{CMY} genes in *Escherichia coli* and Salmonella isolates from food animals and ground meat. *Antimicrob Agents Chemother*, 45, 3647-3650.

HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Sığır Besiciliğinde Performans ve Karkas Değerlerinin Meta Analizi ile İncelenmesi: Türkiye Örneği

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Özet: Bu çalışmada Türkiye'de 2000-2021 yılları arasında sığır besiciliği üzerine yapılan çalışmaların performans ve karkas özellikleri yönünden meta analizi ile incelenerek farklı ırk, bölge, yıl, yaş ve besi süresi için ortak bir sonuca ulaşılması amaçlanmıştır. 2000-2021 yılları arasında yayımlanmış 39 sığır besi çalışmasından elde edilen 136 çalışma bulgusu meta analize dahil edilerek, besi başı canlı ağırlık (BBCA), besi sonu canlı ağırlık (BSCA), günlük canlı ağırlık artışı (GCAA), yemden yararlanma oranı (YYO), sıcak karkas ağırlığı (SIKA), sıcak karkas randımanı (SIKAR), soğuk karkas ağırlığı (SOKA) ve soğuk karkas randımanı (SOKAR) değerlerinin ortalamaları hesaplandı. Toplam heterojenliğin hesaplanmasında Cochran's Q istatistiği, çalışmalar arasındaki varyansın toplam varyansa oranının hesaplanmasında I^2 indisi ve çalışmalar arasındaki gerçek varyansın tahmininde ise τ^2 (tau^2) istatistiği kullanıldı. Çalışma bulgularına göre, en düşük BBCA, BSCA ve GCAA gibi göstergelerde en düşük değerlerin yerli ırklarda (DAK, Boz, Zavot, GAK) ve Doğu Anadolu Bölgesi'nde olduğu tespit edilmiştir. YYO yönünden en düşük ve en yüksek değere sahip alt gruplar sırasıyla; ırklara göre Simental (5.49 kg) ve Yerli ırklar (8.46 kg) (P<0.001); Coğrafi bölgelere göre, Marmara (5.02 kg) ve İç Anadolu (8.23 kg) (P<0.001); Yıllara göre, 2011-2021 (7.35 kg) ve 2000-2010 (7.49 kg) (P=0.750). Besiye alınan yaşa göre, ≥6 ay (6.72 kg) ve ≥18 ay (9.83 kg) (P=0.747). Besi süresine göre, ≥270 gün (6.91 kg) ve ≤180 (7.80 kg) (P=0.154) olduğu tespit edilmiştir. SIKA yönünden en yüksek ağırlıklar Simental (327 kg) ırkı bulunmuştur. SIKAR yönünden en yüksek oranlar Kültür Melezi (%58.6) ırklarında bulunurken, bölgesel olarak incelendiğinde Karadeniz Bölgesi (%60.1) ön plana çıkmaktadır. SOKA yönünden en yüksek ağırlıklar Limuzin (363.7 kg) ırkında, bölgesel olarak ise Karadeniz Bölgesinde (316.6 kg) bulunmuştur. SOKAR yönünden en yüksek oranlar Limuzin (%58.3) ırkında hesaplanmıştır. Sonuç olarak, yapılan bu çalışma Türkiye şartlarında sığır besiciliği yapan/yapacak olan üreticiler ile politikacılara gelecekte sürdürülebilir ve daha kârlı bir üretim yapılması için karar desteği sağlayacaktır.
Anahtar Kelimeler: *Besi sığırı, Karkas, Kârlılık, Meta analiz, Türkiye.*

Meta-Analysis of Performance and Carcass Values in Cattle Fattening: The Case of Turkey

Abstract: This study aimed to conduct a meta-analysis of studies conducted on cattle farming in Türkiye between 2000 and 2021, focusing on performance and carcass characteristics, to reach a common conclusion for different breeds, regions, years, ages, and feeding durations. A total of 136 study results from 39 beef cattle fattening studies published between 2000 and 2021 were included in the meta-analysis. The mean values were calculated that the beginning of fattening body weight (BBCA), end of fattening body weight (BSCA), daily body weight gain (GCAA), feed conversion ratio (YYO), hot carcass weight (SIKA), hot carcass yield (SIKAR), cold carcass weight (SOKA) and cold carcass yield (SOKAR). Cochran's Q statistic was used to calculate total heterogeneity, the I^2 index was used to calculate the ratio of the variance between studies to the total variance, and the τ^2 (tau^2) statistic was used to estimate the actual variance between studies. According to the study results, it was determined that the lowest values for indicators such as BBCA, BSCA, and GCAA were observed in native breeds (DAK, Boz, Zavot, GAK) and the Eastern Anatolia Region. In terms of YYO, the subgroups with the lowest and highest values were, respectively, by breed, Simental (5.49 kg) and Native breeds (8.46 kg) (P<0.001); by geographical region, Marmara (5.02 kg) and Central Anatolia (8.23 kg) (P<0.001); by years, 2011-2021 (7.35 kg) and 2000-2010 (7.49 kg) (P=0.750). According to the age at the start of feeding, ≥6 months (6.72 kg) and ≥18 months (9.83 kg) (P=0.747). In terms of feeding duration, ≥270 days (6.91 kg) and ≤180 days (7.80 kg) (P=0.154) were determined. The highest weights in SIKA were found in the Simental breed (327 kg). The highest proportions in SIKAR were found in crossbred (58.6%) breeds, and when analyzed regionally, the Black Sea Region (60.1%) stood out. Regarding SOKA, the highest weights were in the Limousin breed (363.7 kg); regionally, it was found in the Black Sea Region (316.6 kg). The highest proportions in SOKAR were calculated in the Limousin breed (58.3%). In conclusion, this study will provide decision support to cattle farmers in Türkiye and policymakers, enabling them to make informed decisions for sustainable and more profitable cattle farming practices in the future.
Keywords: *Carcass, Fattening cattle, Meta analysis, Profitability, Türkiye.*

Giriş

Sığır besiciliği, kasaplık olarak beslenen sığırlardan optimum verim kabiliyetlerini göstermesi için gerekli koşullar sağlanarak yüksek miktarda kaliteli et ve yağ elde edilmesini hedefleyen önemli bir hayvansal üretim faaliyetidir. Kırmızı et üretimi, insanların hayvansal protein ihtiyacını karşılamasının yanında endüstriyel kalıntıların değerlendirilmesi, istihdam alanı oluşturması ve milli geliri artırması nedeniyle ulusal ekonomiye de katkı sağlamaktadır (Sakarya ve Günlü, 1996). İkame ürün yönünden kısıtlı bir gıda olan kırmızı et, Türkiye'de büyük oranda sığırlardan elde edilmektedir. Resmi verilere göre, 2021 yılında Türkiye'de toplam kesilen hayvan sayısının (27.2 milyon baş) %18'ini (5.1 milyon baş) oluşturan sığırlar, toplam et üretiminin ise (2 milyon ton) yaklaşık %75'ini (1.5 milyon ton) oluşturmaktadır (HAYGEM, 2022).

Ekonomik bir üretim faaliyeti olan sığır besiciliği, yetiştiricilik ve performans gibi teknik unsurlara da dikkat edilerek yapılmalıdır. Bu doğrultuda sığır besiciliğini; ırk, yaş, cinsiyet, kondisyon, orijin, bakım koşulları, sağlık durumu, besleme stratejisi ve besi süresi doğrudan etkilemektedir (Tuncer, 1984). Üreticiler, mevcut unsurlara optimum düzeyde dikkat ederek minimum maliyet ile maksimum besi performansı ve karkas verimi elde etmeyi hedeflemektedir.

Türkiye'de uzun yıllardır sığır besiciliği üzerine farklı ırk, bölge, zaman, yaş ve besi sürelerinde yapılan çok sayıda çalışma bulunmaktadır (Aydın ve Demirkol, 2021; Aygül ve Özkütük, 2012; Duru ve Sak, 2017; Gözener ve Sayılı, 2015; Kızıl ve Aydoğan, 2014). Fakat belirli bir konu üzerinde birbirinden bağımsız olarak yapılan bireysel çalışmalardan elde edilen bulgular farklı olabilmektedir. Mevcut çalışmada, gerek çok sayıda literatürdeki bilgilerin bir arada değerlendirilip yorumlanması, yeni bilgiler elde edilmesi ve gerekse çalışmalar arasındaki heterojenliklerin giderilerek daha kapsayıcı ve güvenilir nitelikte ortak bir sonuca ulaşılması için meta analiz iyi bir seçenek olarak karşımıza çıkmaktadır (Akgöz ve ark., 2004).

Meta analizi, belirli bir konuda birbirinden bağımsız olarak yapılmış olan çalışma sonuçlarının belirli bir metodoloji ile birleştirilerek ortak bir yargıya ulaşma sürecidir. Meta analizi ile küçük örnekleme sahip çalışmaların sonuçları bilimsel yöntemlerle bir araya getirilerek daha büyük örneklemler ile gücü ve kesinliği daha yüksek parametre kestirimleri yapılabilmektedir (Borenstein ve Higgins, 2013; Hedges ve Tripton, 2010).

Mevcut çalışmada Türkiye'de 2000-2021 yılları arasında sığır besiciliği üzerine yapılan çalışmaların performans ve karkas özellikleri yönünden meta analizi ile incelenerek farklı ırk, bölge, yıl, yaş ve besi süresi için ortak bir sonuca ulaşılması amaçlanmıştır.

Materyal ve Metot

Çalışmanın materyalini, literatürde yer alan besi sığırlarında performans ve karkas özelliklerinin araştırıldığı çalışmaların araştırma bulguları oluşturmuştur. Analiz verilerinin literatürde yer alan açık erişimli tez ve makalelerden temin edilmesi nedeniyle etik izne tabi değildir. Literatür taraması "Sığır, Besicilik, Karkas, Performans ve Türkiye" anahtar kelimeleri kullanılarak Google Akademik, Science Direct ve Scopus veri tabanlarından yapılmıştır. Meta analize dahil etme kriteri olarak çalışmaların; 2000-2021 yılları arasında Türkiye'de yapılmış olması ve besi başı canlı ağırlığı (BBCA), besi sonu canlı ağırlığı (BSCA), günlük canlı ağırlığı artışı (GCAA), yemden yararlanma oranı (YYO), sıcak karkas ağırlığı (SIKA), sıcak karkas randımanı (SIKAR), soğuk karkas ağırlığı (SOKA), soğuk karkas randımanı (SOKAR) değerlerinden en az birini belirtmiş olması koşulu aranmıştır. Bu doğrultuda 597 özet taranarak, kriterlere uyan 39 çalışmadan elde edilen 136 araştırma bulgusu meta analize dahil edilmiştir (Page ve ark., 2021). Meta analizinde kullanılan kaynaklar Tablo 1'de verilmiştir.

Tablo 1. Meta analizinde kullanılan kaynaklar.

1. Aksoy A R, Kirmizibayrak T, Saatci M, 2006: The effect of age on slaughter and carcass characteristics in male Zavot cattle. *Turk J Vet Anım Scı*, 30 (6), 527-532.
2. Alcicek A, Onenc A, Gungor M, 2003: Carcass and meat quality of friesian, piemontese x friesian and limousin x friesian young bulls under intensive beef production system in Turkey. *J Anim Feed Sci*, 12 (2), 249-260.
3. Altuntas M, Arpacık R, 2004: Fattening performance and optimum slaughter weights of Simmental bulls in different starting ages. *Lalahan Hay Araşt Enst Derg,* 44(1), 7-16.
4. Ardıclı S, Dıncel D, Balcı F, 2018: Evaluation of slaughter weights and carcass traits of bulls marketed in South Marmara Region of Turkey. *Harran Üniv Vet Fak Derg*, 7 (1), 45-50.
5. Arıkan MS, Gökhan E, 2018: Limuzin ırkı sığırlarda besi başlangıcındaki canlı ağırlığın ekonomik açıdan besi performansına etkisi. *EJVS*, 34 (4).
6. Aslan E, 2009: Orta Anadolu şartlarında açıkta besiye alınan siyah alaca, esmer ve simental ırkı sığırların besi performanslarının karşılaştırılması. Yüksek lisans tezi, SÜ Sağlık Bilimleri Enstitüsü, Konya.
7. Ayaşan T, Gök K, Asarkaya A, Hizli H, Görgül M, Karakozak E, Seğmenoğlu MS, 2012: Mısır silajı ve şeker pancarı posasının erkek danaların besi performansı, kan parametresi ile kesim ölçütleri üzerine etkisi. *Süleyman Demirel Univ Ziraat Fak Derg*, 7(1), 64-73.
8. Bozkurt Y, Dogan C, 2016: Physıcal performance and carcass characterıstıcs of holstein and brown swıss cattle grown ın an ıntensıve beef system. *Scı Papers*, 75.
9. Çatıkkaş E, Koç A, 2017: Fattening performance, carcass characteristics and beef quality of holstein–friesian, brown–swiss and simmental bulls. *Adü Ziraat Derg*, 14 (1), 59-64.
10. Diler A, Kocyigit R, Yanar M, Aydin R, Tuzemen N, 2016; Effects of different initial weights on fattening performance, slaughter and carcass characteristics of holstein friesian and brown swiss young bulls. *Indian J Anim Res*, 50 (1), 112-117.
11. Duru S, Sak H, 2017: Türkiye 'de besiye alınan simmental, aberdeen angus, hereford, limousin ve charolais ırkı sığırların besi performansı ve karkas özellikleri. *TURJAF*, 5 (11), 1383-1388.

12. Ekiz B, Kocak O, Yilmaz A, 2005: Certain fattening and slaughter characteristics of Holstein, Brown Swiss and Simmental young bulls in the southeastern anatolian region of Turkey. *Bulg J Agric Sci*, 11, 199-206.
13. Ekiz B, Koçak Ö, Yılmaz A, Güneş H, 2004: Certain fattening and slaughter characteristics of Brown Swiss young bulls in intensive conditions. *Istanbul Univ Vet Fak Derg*, 31 (1), 119-128.
14. Garip M, Akmaz A, Yilmaz A, Dere S, Caglayan T, Inal S, Inal F 2010: Determination of optimum slaughter weight and profitability of Brown Swiss cattle in Turkey. *J Food Agric Environ*, 8 (3-4), 864-868.
15. Güngör M, Alçiçek A, 2004: Siyah alaca ve farklı etçi ırk melezlerinin besi performanslarının araştırılması. *ETAE*, 14(1).
16. Kızıl SH, Aydoğan M, 2014: Evaluation of major cattle breeds in Turkey for slaughter and carcass traits using manova and multidimensional scaling technique. *Erciyes Üniv Vet Fak Derg*, 11 (1), 15-22.
17. Koç A, Akman N, 2003: Farklı ağırlıkta besiye alınan ithal edilmiş Siyah-Alaca tosunların besi gücü ve karkas özellikleri. *Hay Üret*, 44 (1), 26-36.
18. Koçak O, Ekiz B, Yilmaz A, 2005: Certain fattening and slaughter characteristics of Simmental young bulls. *Indian Vet J*, 82 (6), 646.
19. Konac V, Akbaş A, Saatcı M, 2019: The effects of drinking water treated with energized oxygen on fattening performance in beef cattle. *Harran Üniv Vet Fak Derg*, 8 (2), 236-242.
20. Kök S, Soysal Mİ, Gürcan EK, 2012: An investigation on the carcass percentage of Anatolian Grey breed in Edirne province. *J Agric Sci Technol*, 2(9A), 1107.
21. Önenç A, 2003: Siyah Alaca, Piedmont X Siyah Alaca, Limuzin X Siyah Alaca tosunlarda etlenme ve yağlanma durumunun karşılaştırılması üzerine bir araştırma. *Hay Üret*, 44 (1), 52-58.
22. Önenç A, 2004: A comparison of Holstein Friesian, Brown Swiss And Eastern Anatolian Red Cattle slaughtered in Turkey for carcass conformation and fatness in seurop system. *Czech J Anim Sci*, 49 (4), 169-176.
23. Özdoğan M, Birincioğlu B, Önenç A, Metin K, 2005: Sığır besi rasyonlarında kullanılan farklı yağ kaynaklarının etkileri: ı. besi performansı. *Adü Ziraat Derg*, 2 (1), 67-72.
24. Özlütürk A, Tüzemen N, Yanar M, Esenbuga N, Dursun E, 2004: Fattening performance, carcass traits and meat quality characteristics of calves sired by Charolais, Simmental and Eastern Anatolian Red Sires Mated to Eastern Anatolian Red Dams. *Meat Sci*, 67 (3), 463-470.
25. Özlütürk A, Esenbuğa N, Yanar M, Uenlue N, Macit M, Kopuzlu S, 2008: The effect of duration of finishing period on the performance, slaughter, carcass, and beef quality characteristics of Eastern Anatolian Red bulls. *Turkish J Vet Anim Sci*, 32 (6), 441-448.
26. Pınarbaşı A, Yazgan K, 2020: Şanlıurfa ilinde besiye alınmış farklı sığır ırklarının besi performanslarının ve karkas özelliklerinin karşılaştırılması. *Harran Tarım ve Gıda Bil Derg*, 24 (2), 212-221.
27. Sağsöz Y, Çoban Ö, Laçin E, Sabuncuoğlu N, Yıldız A, 2005: Esmer ve Şarole X Esmer danaların besi performansı ve karkas özellikleri. *Atatürk Üniv Ziraat Fak Derg, 36*(2), 163-169.
28. Sarıözkan S, Akcay A, Bayram D, 2013: Zavot ırkı sığırlarda karkas özellikleri ve karkas parçalamanın ekonomik yönü. *Ankara Üniv Vet Fak Derg*, 60, 257-262.
29. Soysal D, 2012: Bozırk sığırlarda besi performansı, karkas özellikleri ve et kalitesinin belirlenmesi. Doktora tezi, NKÜ Fen Bil Ens, Tekirdağ.
30. Şahin, A, Miran B, Yildirim I, Önenç A, Alcicek A, 2009: Fattening costs of beef breeds reared under controlled conditions and the determination of optimum fattening period. *Turkish J Vet Anim Sci*, 33 (6), 485-492.
31. Şeker İ, Bayraktar M, Kul S, Yüce M, Esen F, 2001: Farklı sürelerde beslenen Doğu Anadolu Kırmızısı erkek danaların besi, kesim ve karkas özellikleri. *Hay Araş Derg*, 11 (2), 32-37.
32. Şenyüz H, Erat S, Karslı M A, Soydemir İ, 2020: Comparison of fattening performance of Angus, Charolais, Limousine And Simmental cattle imported to Turkey *Lalahan Hay Araşt Enst Derg*, 60 (1), 1-4.
33. Tunçer H, Özbeyaz C, 2009: The effect of restricted feeding in different duration on fattening performance, slaughtering-carcass characteristics and fattening cost on Holstein cattle after puberty. *Lalahan Hay Araşt Enst Derg,* 49 (1), 1-15.
34. Ünlü N, Yanar M, Esenbuga N, Özlütürk A, Yüksel S, Macit M, 2008: Effect of days on feed on the performance and carcass characteristics of Eastern Anatolian Red young bulls. *J Appl Anim Res*, 34 (2), 163-168.
35. Üstuner H, Ardicli S, Arslan O, Brav FC, 2020: Fattening performance and carcass traits of imported simmental bulls at different initial fattening age large. *Anim Rev*, 26 (4), 161-165.
36. Üstuner H, Yalcintan H, Orman A, Ardicli S, Ekiz B, Gencoglu H, Kandazoglu O, 2017: Effects of initial fattening age on carcass characteristics and meat quality in Simmental bulls imported from Austria to Turkey. *S Afr J Anim Sci*, 47 (2), 194-201.
37. Yerturk M, Kaplan O, Avci M, 2011: Fattening performance and dressing percentage of holstein crossbred bulls at different initial weights in Southeastern Anatolia region. *J Anim Vet Adv*, 10 (5), 606-609.
38. Yüksel S, 2019: Effects of housing condition, feeding style, and age on fattening performances, comfort, and some slaughterhouse characteristics in Eastern Anatolian Red bulls. *Turkish J Vet Sci*, 43 (5), 650-655.
39. Yüksel S, Yanar M, Turgut L, Özlütürk A, Kopuzlu S, Sezgin E, 2009: Feed efficiency and carcass and meat quality characteristics of bulls finished on diets containing varied proportions of wheat straw and wet sugar beet pulp. *S Afr J Anim Sci*, 39 (4).

Çalışma kapsamında, Türkiye'de 2000-2021 yılları arasında sığır besiciliği üzerine yapılan çalışmaların araştırma bulgularının meta analizi yapılarak; BBCA, BSCA, GCAA, YYO, SIKA, SIKAR, SOKA ve SOKAR değerlerinin ortalamaları ve %95 güven aralıkları hesaplandı. Toplam heterojenliğin hesaplanmasında Cochran's Q istatistiği, çalışmalar arasındaki varyansın toplam varyansa oranının hesaplanmasında I^2 indisi ve çalışmalar arasındaki gerçek varyansın tahmininde ise τ^2 (tau^2) istatistiği kullanıldı (Baujat ve ark., 2002). Çalışmalar arasındaki heterojenlikten dolayı Rastgele Etki Modeli ve REML yöntemi (Restricted Maximum Likelihood) kullanıldı. Çalışma örneklemindeki yayın yanlılıklarını belirlemek için Duval ve Tweedie'nin kırp ve doldur yöntemi ve huni grafiği kullanıldı. Moderatör değişkenler; besiye alınan hayvanın ırkı ve yaşı, araştırmanın yapıldığı yıl ve coğrafi bölge ile besi süresi olarak beş kategori olarak belirlendi. Irk değişkeninin alt kategorileri; Şarole, Holstein, İsviçre Esmeri, Angus, Brangus, Hereford, Limuzin, Simental Kültür Melezi (Angus+Brangus, Holstein+İsviçre Esmeri, Holstein+İsviçre Esmeri+Simental, Piedmont+Holstein), ve Yerli ırklar (Boz, Güney Anadolu Kırmızısı, Doğu Anadolu Kırmızısı, Zavot) olarak; yıl değişkeninin alt kategorileri ise 2000-2010 ve 2011-2021 olarak ve bölge değişkeni Ege, Karadeniz, Doğu Anadolu, Marmara, Akdeniz, İç Anadolu ve Güney Doğu Anadolu Bölgesi, yaş değişkeni ≤6 ay, 7-17 ay ve ≥18 ay; besi süresi ise kısa (≤180 gün), orta (181-269 gün) ve uzun (≥270 gün) olarak belirlendi. Alt grup analizlerinde, alt grup oluşturmak için gerekli sayıyı sağlayamayan kategoriler veri setinden çıkarıldı. Ortak etki büyüklüklerinin anlamlılık düzeyi $P<0.05$, Cochran's Q istatistiğinin anlamlılık düzeyi $P<0.10$ olarak belirlendi. Meta-analizlerde Stata/SE 17.0 istatistiksel yazılımı kullanıldı.

Bulgular

Yapılan meta analizde besi performansı (BBCA, BSCA, GCAA, YYO) ve karkas verimine (SIKA, SIKAR, SOKA, SOKAR) ait elde edilen değerlerin ortalamaları ve analize dahil edilen hayvan sayıları ile diğer istatistik bilgiler Tablo 2'de verilmiştir.

Tablo 2. Besi performansı ve karkaslara ilişkin ortalamaların meta analiz sonuçları.

Değişken	Ortak Ortalama (%95 GA)	Hayvan Sayısı	z Değeri	P Değeri	Cochran's Q	sd	I^2	P Değeri (Cochrane's Q)	τ^2
BBCA, kg	226.78 (213.53-240.02)	4734	33.55	<0.001	47024.16	105	99.84	<0.001	4.8e+03
BSCA, kg	473.41 (453.49-493.34)	7463	46.57	<0.001	50991.11	117	99.87	<0.001	1.2e+04
GCAA, g	1100.90 (1057.23-1144.57)	4734	49.41	<0.001	2.1e+09	105	100.00	<0.001	5.2e+04
YYO, kg	7.43 (7.05-7.81)	663	38.21	<0.001	2101.55	51	99.82	<0.001	1.87
SIKA, kg	265.23 (252.39-278.07)	6716	40.48	<0.001	57608.12	108	99.85	<0.001	4.6e+03
SIKAR, %	56.94 (56.33-57.54)	8112	18446	<0.001	29512.45	109	99.85	<0.001	10.11
SOKA, kg	276.68 (260.65-292.71)	2339	33.84	<0.001	9046.71	53	99.58	<0.001	3.6e+03
SOKAR, %	55.13 (54.28-55.97)	2579	127.77	<0.001	16549.82	50	99.93	<0.001	9.18

GA: Güven aralığı; Sd: Serbestlik derecesi; I^2:Gerçek varyansın gözlemlenen varyansa oranı; Cochran's Q: Gözlemlenen etki büyüklüklerinin ağırlıklı kareler toplamı.

Yapılan meta-analiz sonuçlarına göre tüm değişkenler için çalışmalar arasında yüksek bir heterojenlik olduğu görülmüştür. Ortalama ve %95 güven aralığında BBCA 226.78 kg (213.53-240.02 kg), BSCA 473.41 kg (453.49-493.34 kg), GCAA 1100.90 g (1057.23-1144.57 g); YYO 7.43 kg (7.05-7.81 kg); SIKA 265.23 kg (252.39-278.07 kg), SIKAR % 56.94 (%56.33-%57.54); SOKA 276.68 kg (260.65-292.71 kg) ve SOKAR %55.13 (%54.28-%55.97) olarak bulunmuştur (P<0.001; Tablo 2).

Çalışmada heterojenliğin kaynaklarını saptamak için performans (BBCA, BSCA, GCAA, YYO) ile sıcak/soğuk karkas ağırlık (SIKA, SOKA) ve randımanına (SIKAR, SOKAR) ait değişkenlerin yıllara, bölgelere, besiye alınan yaşa, ırklara ve besi süresine göre alt grup analizleri yapılarak sırasıyla Tablo 3-6'da verildi.

BBCA için yapılan alt grup analizinde ırk, coğrafi bölge, yıl, besiye alınan yaş ve besi süresi yönünden alt gruplar arasında anlamlı bir farklılık olduğu görülmüştür (P<0.05). BBCA yönünden en düşük ve en yüksek değere sahip alt gruplar sırasıyla; ırklara göre Yerli ırklar (164.63 kg) ve Limuzin (294.08 kg) (P<0.001); coğrafi bölgelere göre, Marmara (193.68 kg) ve Karadeniz (286.77 kg) (P<0.001); yıllara göre, 2000-2010 (210.94 kg) ve 2011-2021 (240.72 kg) (P=0.024); besiye alınan yaşa göre, ≤6 ay (185.02 kg) ve ≥18 ay (271.07 kg) (P<0.001); besi süresine göre, ≥270 (198.48 kg) ve ≤180 gün (245.78 kg) (P<0.001) olarak hesaplandı (Tablo 3).

BSCA için yapılan alt grup analizinde ırk, coğrafi bölge, yıl, besiye alınan yaş ve besi süresi yönünden alt gruplar arasında anlamlı bir farklılık olduğu görülmüştür (P<0.05). BSCA yönünden en düşük ve en yüksek değere sahip alt gruplar sırasıyla; ırklara göre Yerli ırklar (319.19 kg) ve Limuzin (610.15 kg) (P<0.001); coğrafi bölgelere göre, Doğu Anadolu (368.12 kg) ve Karadeniz (586.07 kg) (P<0.001); yıllara göre, 2000-2010 (434.72 kg) ve 2011-2021 (508.21 kg) (P=0.024); besiye alınan yaşa göre, ≥18 ay (395.11 kg) ve ≤6 ay (494.48 kg) (P<0.001); besi süresine göre, ≤180 gün (408.84 kg) ve ≥270 (516.21 kg) (P<0.001) olarak hesaplandı (Tablo 3).

GCAA için yapılan alt grup analizinde ırk ve coğrafi bölge alt grupları için anlamlı bir farklılık gösterirken (P<0.05), yıl, besiye alınan yaş ve besi süresi yönünden alt gruplar arasında anlamlı bir farklılık bulunamamıştır (P>0.05). GCAA yönünden en düşük ve en yüksek değere sahip alt gruplar sırasıyla; ırklara göre Yerli ırklar (771.32 g) ve Simental (1308.42 g) (P<0.001); Coğrafi bölgelere göre, Doğu Anadolu (923.95 g) ve Güneydoğu Anadolu (1254.41 g) (P<0.001); Yıllara göre, 2000-2010 (1064.88 g) ve 2011-2021 (1132.82 g) (P=0.127); Besiye alınan yaşa göre, ≥18 ay (989.83 g) ve ≤6 ay (1072.72 g) (P=0.747); Besi süresine göre, ≤180 gün (1091.15 g) ve 181-269 (1121.99 g) (P=0.829) olarak hesaplandı (Tablo 4).

YYO için yapılan alt grup analizinde ırk, coğrafi bölge ve besiye alınan yaş alt grupları için anlamlı bir farklılık gösterirken (P<0.05), yıl ve besi süresi yönünden alt gruplar arasında anlamlı bir farklılık bulunamamıştır (P>0.05). YYO yönünden en düşük ve en yüksek değere sahip alt gruplar sırasıyla; ırklara göre Simental (5.49 kg) ve Yerli ırklar (8.46 kg) (P<0.001); coğrafi bölgelere göre, Marmara (5.02 kg) ve İç Anadolu (8.23 kg) (P<0.001); yıllara göre, 2011-2021 (7.35 kg) ve 2000-2010 (7.49 kg) (P=0.750); besiye alınan yaşa göre, ≥6 ay (6.72 kg) ve ≥18 ay (9.83 kg) (P=0.747); besi süresine göre, ≥270 gün (6.91 kg) ve ≤180 (7.80 kg) (P=0.154) olarak hesaplandı (Tablo 4).

SIKA için yapılan alt grup analizinde ırk, coğrafi bölge, yıl ve besi süresi alt grupları için anlamlı bir farklılık gösterirken (P<0.05), besiye alınan yaş yönünden alt gruplar arasında anlamlı bir farklılık bulunamamıştır (P>0.05). SIKA yönünden

Tablo 3. BBCA ve BSCA'nın ırk, bölge, yıl, yaş ve besi süresine göre alt grup analizi.

Değişkenler	BBCA (kg)			BSCA (kg)		
	Ortak Ortalama (%95 GA)	Sd	I^2	Ortak Ortalama (%95 GA)	Sd	I^2
Irk Alt Grupları						
Şarole	241.79 (178.45-305.12)	6	99.75	452.86 (360.31-545.40)	6	99.54
Holstein	254.17 (224.04-284.31)	22	99.90	486.28 (463.57-508.98)	25	99.43
İsviçre Esmeri	231.80 (208.50-255.10)	16	99.00	502.95 (467.05-538.85)	19	99.91
Kültür Melezi	210.90 (193.78-228.01)	14	99.11	477.71 (438.93-516.48)	15	99.38
Limuzin	294.08 (245.54-342.63)	4	99.88	610.15 (575.29-645.02)	4	99.60
Simental	238.65 (202.34-274.95)	19	99.90	568.37 (549.24-587.51)	20	98.15
Yerli Irklar	164.63 (147.46-182.10)	18	96.34	319.19 (288.45-349.94)	22	98.79
Genel	226.78 (213.53-240.02)	105	99.84	473.41 (453.49-493.34)	117	99.87
Anlamlılık	Cochran's Q (sd=6) = 52.40, P<0.001			Cochran's Q (sd=6) = 223.14, P<0.001		
Bölge Alt Grupları						
Ege	257.02 (228.25-285.79)	12	99.72	522.42 (506.84-537.99)	12	96.47
Karadeniz	286.77 (171.68-401.86)	5	99.68	586.07 (542.20-629.95)	5	96.04
Doğu Anadolu	195.42 (177.47-213.37)	35	99.27	368.12 (333.85-402.38)	38	99.74
Marmara	193.68 (178.68-208.68)	7	83.25	529.59 (484.59-574.59)	13	99.52
Akdeniz	266.71 (173.75-359.67)	5	99.90	514.17 (460.90-567.45)	5	99.70
İç Anadolu	263.92 (249.72-278.11)	18	98.64	540.08 (514.05-566.11)	18	99.13
Güneydoğu Anadolu	208.24 (182.64-233.84)	17	99.91	497.37 (469.22-525.52)	20	99.87
Genel	226.78 (213.53-240.02)	105	99.84	473.41 (453.49-493.34)	117	99.87
Anlamlılık	Cochran's Q (sd=6) = 64.29, P<0.001			Cochran's Q (sd=6) = 86.47, P<0.001		
Yıl Alt Grupları						
2000-2010	210.94 (193.05-228.84)	49	99.79	434.72 (405.03-464.41)	55	99.90
2011-2021	240.72 (222.06-259.37)	55	99.85	508.21 (484.35-532.08)	61	99.77
Genel	226.78 (213.53-240.02)	105	99.84	473.41 (453.49-493.34)	117	99.87
Anlamlılık	Cochran's Q (sd=1) = 5.10, P=0.024			Cochran's Q (sd=1) = 14.30, P<0.001		
Yaş (ay) Alt Grupları						
≤6	185.02 (169.89-200.15)	16	99.09	494.48 (454.13-534.83)	17	99.59
7-17	228.00 (210.03-245.97)	62	99.78	454.53 (422.37-486.68)	62	99.84
≥18	271.07 (233.29-308.84)	5	90.61	395.11 (332.24-457.99)	7	98.10
Genel	222.34 (207.91-236.77)	85	99.76	457.47 (432.26-482.67)	88	99.80
Anlamlılık	Cochran's Q (sd=2) = 24.30, P<0.001			Cochran's Q (sd=2) = 7.01, P=0.030		
Besi Süresi (gün) Alt Grupları						
Kısa (≤180)	245.78 (219.26-272.31)	37	99.86	408.84 (370.87-446.82)	40	99.76
Orta (181-269)	243.76 (217.44-270.08)	26	99.67	493.96 (454.56-533.36)	26	99.66
Uzun (≥270)	198.48 (185.41-211.54)	40	99.69	516.21 (492.32-540.10)	40	99.69
Genel	226.78 (213.53-240.02)	105	99.84	470.36 (449.00-491.72)	108	99.80
Anlamlılık	Cochran's Q (sd=2) = 15.84, P<0.001			Cochran's Q (sd=2) = 22.13, P<0.001		

BBCA: Besi başı canlı ağırlık; BSCA: Besi sonu canlı ağırlık; GA: Güven aralığı; Sd: Serbestlik derecesi; I^2: Gerçek varyansın gözlemlenen varyansa oranı; Cochran's Q: Gözlemlenen etki büyüklüklerinin ağırlıklı kareler toplamı.

Tablo 4. GCAA ve YYO'nun ırk, bölge, yıl, yaş ve besi süresine göre alt grup analizi.

Değişkenler	GCAA (g)			YYO (%)		
	Ortak Ortalama (%95 GA)	Sd	I^2	Ortak Ortalama (%95 GA)	Sd	I^2
Irk Alt Grupları						
Şarole	1082.35 (973.24-1191.47)	6	93.63	6.71 (6.00-7.44)	3	84.31
Holstein	1144.18 (1065.64-1222.72)	22	100.00	7.78 (7.01-8.54)	7	96.81
İsviçre Esmeri	1171.23 (1102.82-1239.64)	16	99.15	7.63 (7.05-8.21)	11	93.28
Kültür Melezi	1055.82 (996.29-1115.35)	14	97.98	7.02 (6.42-7.61)	6	92.97
Limuzin	1277.13 (1246.72-1307.53)	4	54.21	6.47 (6.29-6.64)	2	99.39
Simental	1308.42 (1237.74-1379.11)	19	100.00	5.49 (4.62-6.36)	4	92.85
Yerli Irklar	771.32 (716.26-826.38)	18	100.00	8.46 (7.59-9.34)	12	97.07
Genel	1100.90 (1057.23-1144.57)	105	100.00	7.43 (7.05-7.81)	51	99.82
Anlamlılık	Cochran's Q (sd=6) = 279.27, P<0.001			Cochran's Q (sd=6) = 48.27, P<0.001		
Bölge Alt Grupları						
Ege	1248.54 (1137.01-1360.07)	12	99.70	6.63 (6.29-6.96)	5	71.20
Karadeniz	1179.56 (1129.90-1229.21)	5	65.14	-	-	-
Doğu Anadolu	923.95 (854.36-993.53)	35	100.00	7.76 (7.29-8.23)	32	99.88
Marmara	1143.66 (940.58-1346.73)	7	100.00	5.02 (4.74-5.30)	3	0.00
Akdeniz	1205.91 (1046.51-1365.30)	5	98.21	7.35 (6.84-7.85)	3	92.66
İç Anadolu	1117.96 (1057.80-1178.11)	18	100.00	8.23 (7.21-9.24)	4	94.87
Güneydoğu Anadolu	1254.41 (1190.13-1318.68)	17	98.13	-	-	-
Genel	1100.90 (1057.23-1144.57)	105	100.00	7.43 (7.05-7.81)	51	99.82
Anlamlılık	Cochran's Q (sd=6) = 56.40, P<0.001			Cochran's Q (sd=4)= 151.36, P<0.001		
Yıl Alt Grupları						
2000-2010	1064.88 (1000.57-1129.20)	49	100.00	7.49 (7.11-7.86)	28	93.10
2011-2021	1132.82 (1073.98-1191.66)	55	100.00	7.35 (6.63-8.08)	22	99.95
Genel	1100.90 (1057.23-1144.57)	105	100.00	7.43 (7.05-7.81)	51	99.82
Anlamlılık	Cochran's Q (sd=1) = 2.33, P=0.127			Cochran's Q (sd=1) = 0.10, P=0.750		
Yaş (ay) Alt Grupları						
≤6	1072.72 (1008.25-1137.19)	16	100.00	6.72 (5.90-7.54)	8	97.18
7-17	1050.80 (994.68-1106.91)	62	100.00	7.25 (6.91-7.60)	35	99.78
≥18	989.83 (753.79-1225.87)	5	100.00	9.83 (8.61-11.05)	5	89.53
Genel	1050.80 (1005.22-1096.39)	85	100.00	7.44 (7.05-7.83)	50	99.83
Anlamlılık	Cochran's Q (sd=2) = 0.58, P=0.747			Cochran's Q (sd=2) = 18.33, P<0.001		
Besi Süresi (gün) Alt Grupları						
Kısa (≤180)	1091.15 (995.49-1186.81)	37	100.00	7.80 (7.23-8.36)	25	97.45
Orta (181-269)	1121.99 (1045.77-1198.21)	26	100.00	7.40 (6.95-7.86)	8	86.64
Uzun (≥270)	1095.49 (1044.22-1146.77)	40	100.00	6.91 (6.21-7.62)	16	99.95
Genel	1100.90 (1057.23-1144.57)	105	100.00	7.43 (7.05-7.81)	51	99.82
Anlamlılık	Cochran's Q (sd=2) = 0.38, P=0.829			Cochran's Q (sd=2)= 3.74, P=0.154		

GCAA: Günlük canlı ağırlık artışı; YYO: Yemden yararlanma oranı; GA: Güven aralığı; Sd: Serbestlik derecesi; I^2: Gerçek varyansın gözlemlenen varyansa oranı; Cochran's Q: Gözlemlenen etki büyüklüklerinin ağırlıklı kareler toplamı.

Tablo 5. SIKA ve SIKAR'ın ırk, bölge, yıl, yaş ve besi süresine göre alt grup analizi.

Değişkenler	SIKA (kg)			SIKAR (%)		
	Ortak Ortalama (%95 GA)	Sd	I^2	Ortak Ortalama (%95 GA)	Sd	I^2
Irk Alt Grupları						
Şarole	264.63 (198.89-330.37)	4	99.17	57.44 (56.48-58.40)	4	76.91
Holstein	274.68 (259.56-289.81)	23	99.47	56.03 (54.91-57.15)	23	99.91
İsviçre Esmeri	288.95 (265.62-312.28)	15	99.87	57.31 (56.30-58.32)	19	99.15
Kültür Melezi	280.02 (246.52-313.52)	11	99.43	58.58 (56.91-60.25)	13	98.43
Limuzin	-	-	-	-	-	-
Simental	327.25 (317.45-337.04)	21	98.32	57.73 (56.55-58.91)	21	99.84
Yerli Irklar	193.05 (171.03-215.08)	29	99.57	55.75 (54.02-57.49)	24	99.55
Genel	265.23 (252.39-278.07)	108	99.85	56.94 (56.33-57.54)	109	99.85
Anlamlılık	Cochran's Q (sd=5) = 132.69, P < 0.001			Cochran's Q (sd=5) = 10.51, P=0.062		
Bölge Alt Grupları						
Ege	299.71 (280.77-318.66)	12	99.66	56.98 (55.45-58.51)	12	94.40
Karadeniz	338.33 (312.89-363.77)	4	90.48	60.13 (58.47-61.79)	4	86.87
Doğu Anadolu	197.50 (178.51-216.48)	36	99.44	57.28 (56.11-58.45)	33	99.51
Marmara	291.88 (266.50-317.26)	15	99.46	53.91 (52.56-55.27)	15	99.87
Akdeniz	297.15 (257.95-336.34)	3	98.48	54.07 (52.55-55.59)	3	86.09
İç Anadolu	300.92 (285.33-316.52)	12	96.95	58.83 (57.27-60.40)	16	99.94
Güneydoğu Anadolu	296.75 (276.87-316.63)	20	99.86	56.96 (56.30-57.62)	20	98.93
Genel	265.23 (252.39-278.07)	108	99.85	56.94 (56.33-57.54)	109	99.85
Anlamlılık	Cochran's Q (sd=6) = 106.79, P<0.001			Cochran's Q (sd=6) = 52.76, P<0.001		
Yıl Alt Grupları						
2000-2010	244.49 (225.20-263.77)	49	99.89	57.34 (56.65-58.04)	51	99.77
2011-2021	282.76 (266.76-298.76)	58	99.73	56.58 (55.62-57.54)	57	99.84
Genel	265.23 (252.39-278.07)	108	99.85	56.94 (56.33-57.54)	109	99.85
Anlamlılık	Cochran's Q (sd=1) = 8.96, P = 0.003			Cochran's Q (sd=1) = 1.61, P=0.205		
Yaş (ay) Alt Grupları						
≤6	284.79 (246.49-323.10)	10	99.66	57.44 (55.72-59.16)	12	99.75
7-17	244.67 (223.01-266.33)	48	99.69	57.10 (56.24-57.97)	53	99.86
≥18	245.87 (208.18-283.56)	10	98.30	54.66 (51.51-57.81)	7	97.20
Genel	251.14 (233.88-268.39)	70	99.65	56.91 (56.14-57.68)	74	99.87
Anlamlılık	Cochran's Q (sd=2) = 3.35, P = 0.187			Cochran's Q (sd=2) = 2.42, P=0.298		
Besi Süresi (gün) Alt Grupları						
Kısa (≤180)	228.78 (204.89-252.67)	37	99.86	56.16 (55.52-56.80)	35	92.61
Orta (181-269)	284.51 (256.78-312.24)	21	99.64	58.16 (56.73-59.58)	23	99.93
Uzun (≥270)	291.56 (274.76-308.35)	27	99.37	56.85 (55.86-57.85)	32	99.79
Genel	262.75 (248.00-277.50)	87	99.80	56.92 (56.33-57.50)	92	99.80
Anlamlılık	Cochran's Q (sd=2) = 18.51, P<0.001			Cochran's Q (sd=2) = 6.64, P=0.036		

SIKA: Sıcak karkas ağırlığı; SIKAR: Sıcak karkas randımanı; GA: Güven aralığı; Sd: Serbestlik derecesi; I^2: Gerçek varyansın gözlemlenen varyansa oranı; Cochran's Q: Gözlemlenen etki büyüklüklerinin ağırlıklı kareler toplamı.

en düşük ve en yüksek değere sahip alt gruplar sırasıyla; ırklara göre Yerli ırklar (193.05 kg) ve Simental (327.25 kg) (P<0.001); coğrafi bölgelere göre, Doğu Anadolu (197.50 kg) ve Karadeniz (338.33 kg) (P<0.001); yıllara göre, 2000-2010 (244.49 kg) ve 2011-2021 (282.76 kg) (P=0.003); besiye alınan yaşa göre, 7-17 ay (244.67 kg) ve ≤6 ay (284.79 kg) (P=0.187); besi süresine göre, ≤180 gün (228.78 kg) ve ≥270 gün (291.56 kg) (P<0.001) olarak hesaplandı (Tablo 5).

SIKAR için yapılan alt grup analizinde, coğrafi bölge ve besi süresi alt grupları için anlamlı bir farklılık gösterirken (P<0.05), ırk, yıl, besiye alınan yaş yönünden alt gruplar arasında anlamlı bir farklılık bulunamamıştır (P>0.05). SIKAR yönünden en düşük ve en yüksek değere sahip alt gruplar sırasıyla; ırklara göre Yerli ırklar (%55.75) ve Kültür melezi (%58.58) (P=0.062); coğrafi bölgelere göre, Marmara (%53.91) ve Karadeniz (%60.13) (P<0.001); yıllara göre, 2011-2021 (%56.58) ve 2000-2010 (%57.34) (P=0.205); besiye alınan yaşa göre, ≥ 18 ay (%54.66) ve ≤6 ay (%57.44) (P=0.298); besi süresine göre, ≤180 gün (%56.16) ve 181-269 gün (%58.16) (P=0.036) olarak hesaplandı (Tablo 5).

SOKA için yapılan alt grup analizinde ırk ve coğrafi bölge alt grupları için anlamlı bir farklılık gösterirken (P<0.05), yıl,

Tablo 6. SOKA ve SOKAR'ın ırk, bölge, yıl, yaş ve besi süresine göre alt grup analizi.

Değişkenler	SOKA (kg)			SOKAR (%)		
	Ortak Ortalama (%95 GA)	Sd	I^2	Ortak Ortalama (%95 GA)	Sd	I^2
Irk Alt Grupları						
Şarole	-	-	-	-	-	-
Holstein	267.40 (256.46-278.35)	11	97.59	54.67 (53.30-56.03)	10	99.96
İsviçre Esmeri	299.24 (264.45-334.03)	7	99.38	55.19 (51.09-59.29)	2	97.60
Kültür Melezi	266.50 (236.55-296.46)	7	98.69	55.31 (52.38-58.25)	5	96.16
Limuzin	363.66 (355.39-371.92)	2	70.48	58.26 (57.19-59.34)	2	59.21
Simental	320.83 (312.52-329.14)	12	88.95	56.34 (54.74-57.95)	12	99.88
Yerli Irklar	194.27 (157.14-231.41)	9	98.92	53.89 (52.06-55.71)	12	99.66
Genel	276.68 (260.65-292.71)	53	99.58	55.25 (54.39-56.11)	48	99.93
Anlamlılık	Cochran's Q (sd=5) = 253.52, P<0.001			Cochran's Q (sd=5) = 25.76, P<0.001		
Bölge Alt Grupları						
Ege	292.23 (284.47-299.99)	9	90.49	55.83 (53.62-58.04)	6	95.75
Karadeniz	316.61 (295.68-337.53)	2	74.33	60.44 (59.47-61.41)	2	47.95
Doğu Anadolu	244.82 (205.21-284.42)	16	99.59	55.14 (53.53-56.75)	19	99.73
Marmara	283.56 (258.52-308.60)	13	99.51	58.66 (53.01-54.31)	13	99.32
Akdeniz	-	-	-	-	-	-
İç Anadolu	293.10 (263.45-322.76)	9	99.36	56.26 (55.09-57.44)	4	99.96
Güneydoğu Anadolu	-	-	-	-	-	-
Genel	276.68 (260.65-292.71)	53	99.58	55.25 (54.39-56.11)	48	99.93
Anlamlılık	Cochran's Q (sd = 4) = 11.16, P=0.025			Cochran's Q (sd=4) = 130.14, P<0.001		
Yıl Alt Grupları						
2000-2010	268.08 (235.72-300.43)	17	99.54	55.55 (53.62-57.48)	15	99.98
2011-2021	281.00 (263.04-298.96)	35	99.55	55.11 (54.21-56.01)	32	99.78
Genel	276.68 (260.65-292.71)	53	99.58	55.25 (54.39-56.11)	48	99.93
Anlamlılık	Cochran's Q (sd=1) = 0.47, P=0.494			Cochran's Q (sd=1) = 0.16, P=0.685		
Yaş (ay) Alt Grupları						
≤6	276.82 (248.87-304.76)	15	99.41	55.41 (53.43-57.40)	12	99.83
7-17	296.56 (268.86-324.26)	18	99.50	54.97 (53.48-56.45)	16	99.97
≥18	223.81 (152.10-295.52)	2	99.07	56.32 (54.37-58.27)	2	91.74
Genel	282.53 (262.99-302.07)	37	99.50	55.26 (54.18-56.35)	32	99.95
Anlamlılık	Cochran's Q (sd=2) = 3.72, P=0.156			Cochran's Q (sd=2) = 1.17, P=0.556		
Besi Süresi (gün) Alt Grupları						
Kısa (≤180)	235.87 (186.47-285.27)	7	99.67	53.25 (51.81-54.70)	7	88.39
Orta (181-269)	282.21 (247.90-316.51)	7	99.48	54.88 (53.24-56.53)	6	99.95
Uzun (≥270)	298.42 (278.24-318.59)	24	99.12	55.73 (54.29-57.18)	20	99.92
Genel	282.97 (264.85-301.09)	40	99.48	55.01 (54.01-56.01)	35	99.94
Anlamlılık	Cochran's Q (sd=2) =5.41, P=0.067			Cochran's Q (sd = 2) = 5.82, P=0.055		

SOKA: Soğuk karkas ağırlığı; SOKAR: Soğuk karkas randımanı; GA: Güven aralığı; Sd: Serbestlik derecesi; I^2: Gerçek varyansın gözlemlenen varyansa oranı; Cochran's Q: Gözlemlenen etki büyüklüklerinin ağırlıklı kareler toplamı.

besi süresi ve besiye alınan yaş yönünden alt gruplar arasında anlamlı bir farklılık bulunamamıştır (P>0.05). SOKA yönünden en düşük ve en yüksek değere sahip alt gruplar sırasıyla; ırklara göre Yerli ırklar (194.27 kg) ve Limuzin (363.66 kg) (P<0.001); Coğrafi bölgelere göre, Doğu Anadolu (244.82 kg) ve Karadeniz (316.61 kg) (P=0.025); Yıllara göre, 2000-2010 (268.08 kg) ve 2011-2021 (281.00 kg) (P=0.494); Besiye alınan yaşa göre, ≥ 18 ay (223.81 kg) ve 7-17 ay (296.56 kg) (P=0.156); Besi süresine göre, ≤180 gün (235.87 kg) ve ≥270 gün (298.42 kg) (P=0.067) olarak hesaplandı (Tablo 6).

SOKAR için yapılan alt grup analizinde, ırk ve coğrafi bölge, ve alt grupları için anlamlı bir farklılık gösterirken (P<0.05), yıl, besiye alınan yaş ve besi süresi yönünden alt gruplar arasında anlamlı bir farklılık bulunamamıştır (P>0.05). SOKAR yönünden en düşük ve en yüksek değere sahip alt gruplar sırasıyla; ırklara göre Yerli ırklar (%53.89) ve Limuzin (%58.26) (P<0.001); Coğrafi bölgelere göre, Doğu Anadolu (%55.14) ve Karadeniz (%60.44) (P<0.001); Yıllara göre, 2011-2021 (%55.11) ve 2000-2010 (%55.55) (P=0.685); Besiye alınan yaşa göre, 7-17 ay (%54.97) ve ≥ 18 ay (%56.32) (P=0.556); Besi süresine göre, ≤180 gün (%53.25) ve ≥270 gün (%55.73) (P=0.055) olarak hesaplandı (Tablo 6).

Tartışma ve Sonuç

Son yıllarda artan nüfus ve iklim değişiklikleri ile yaşanan ekonomik gelişmeler (pandemi, enflasyon vs.), hayvansal ürün ihtiyacının miktar ve kalite açısından değişmesine neden olmuştur. Günümüzde hayvansal ürün arz eden işletmeler bir taraftan üretim maliyetlerini düşürmeyi, diğer taraftan ürünlerin niteliğini/niceliğini artırarak rekabette avantajlı konuma gelmeyi hedeflemektedir. Bu nedenle bütün dünyada olduğu gibi Türkiye'de de hayvansal üretim potansiyelinin geliştirilmesi yönünde yapılan çalışmalar hızla artmaktadır. Bu kapsamda çevresel faktörler optimize edilerek genetik yapının güçlendirilmesi ve daha rasyonel besleme stratejileri geliştirilerek verimliliğin artırılması sağlanmaktadır (Sever ve ark., 2017).

Besi sığırcılığında performans ve verimlilik üzerine yapılan çalışmalar incelendiğinde Türkiye'de farklı ırk, coğrafi bölge, yıl, yaş ve besi süresinde yapılmış ayrı ayrı birçok çalışma vardır. Fakat bu çalışmalar arasında mevcut faktörlerden ve örneklem sayısındaki farklılıklardan kaynaklandığı düşünülen heterojenlikler bulunmaktadır. Bu noktada belirli bir konu üstünde birbirinden bağımsız olarak elde edilen çalışma bulgularının yeniden değerlendirilerek yorumlanmasına olanak tanıyan meta analiz, çalışmalar arasındaki heterojenliklerin giderilerek daha güçlü parametre tahminleri yapılmasına imkân sağlamaktadır.

Türkiye'de sayıca azalan yerli ırkların yanında son yıllarda giderek artan kültür ve kültür melezi sığırlarla besi faaliyetleri yürütülmektedir. Ülkemizde ırk kompozisyonu incelendiğinde kültür ve kültür melezi ırkların oranı %92.5'e yükselirken, yerli ırkların oranı %7.5'e kadar gerilemiştir (HAYGEM, 2022). Yapılan besicilikte farklı ırkların (kültür ve melez) Türkiye şartlarına adaptasyonu ve performanslarının kıyaslandığı sınırlı sayıda çalışma bulunmaktadır (Aydın ve Demirkol 2021; Duru ve Sak 2017; Gözener ve Sayılı, 2015; Kızıl ve Aydoğan, 2014). O nedenle bu çalışmada yapılan meta analiz ile Türkiye'de besi sığırcılığının mevcut durumu ve farklı ırklara ait performans (besi ve karkas) göstergeleri detaylı olarak ortaya konulmuş, mukayese edilmesine olanak tanınmış ve üreticilere yapmayı düşündükleri besi öncesi alternatif seçeneklere yönelik karar desteği sağlanmıştır. Hatta belirli dönemlerde mecbur kalınan canlı hayvan ithalatlarında politikacılara da yeni bir bakış açısı sağlayabilecektir.

Yapılan çalışma genel olarak incelendiğinde; en düşük BBCA, BSCA ve GCAA gibi göstergelerde en düşük değerlerin yerli ırklarda (DAK, Boz, Zavot, GAK) ve Doğu Anadolu Bölgesi'nde gerçekleştiği göze çarpmaktadır. Buna göre yerli ırkların ıslahının yapılması ve Doğu Anadolu Bölgesi'nde üreticilerin besicilik konusunda teknik ve işletmecilik düzeyinde eğitilmesinin sektörel anlamda gelişime katkı sağlayacağı düşünülmektedir.

Besicilikte performans denilince teknik olarak GCAA ve YYO kastedilmektedir (Sağsöz ve ark., 2005). Çünkü besi sığırcılığında CAA, YYO arasındaki ilişki karlılığı direk olarak etkilemektedir. Bu iki göstergeye ait sonuçlar incelendiğinde, doğal olarak GCAA yönünden yerli ırkların, kültür ve melezlere oranla daha düşük düzeyde olduğu belirlenirken, Türkiye şartlarında en yüksek GCAA sırasıyla Simental (1308.4 g/gün) ve Limuzin (1277.1 g/gün) ırklarında gerçekleşmiştir. Bölgesel olarak en düşük GCAA Doğu Anadolu Bölgesi'nde (923 g/gün), en yüksek ise Güneydoğu Anadolu Bölgesi'nde (1254.4 g/gün) hesaplanmıştır. Besi süresi yönünden kısa süreli beside (<180 gün) daha yüksek (1072.7 g/gün) GCAA sağlandığı söylenebilir. Çek Cumhuriyeti'nde yapılan çalışmalarda da Şarole ve Simental ırkının GCAA Angus ve Hereford ırklarından daha yüksek olduğu bildirilmiştir (Barton ve ark., 2006; Jakubec ve ark., 2003). Mevcut çalışmadan elde edilen sonuçlara benzer şekilde GCAA en yüksek kısa süreli beside bulunmuştur (Jakubec ve ark., 2003).

Hayvancılıkta YYO düşük olması, işletmelere daha düşük maliyetle daha yüksek kar elde etme olanağı sağlayacaktır. YYO yönünden en düşük ve en yüksek değere sahip alt gruplar sırasıyla; ırklara göre Simental (5.49 kg) ve Yerli ırklar (8.46 kg) (P<0.001) olduğu tespit edilmiştir. YYO bakımından Simental ırkının diğer besi ırklarının (Angus, Şarole, Limuzin, Hereford) gerisinde kaldığı bildirildiği çalışmalar (Laborde ve ark., 2001; Crowley ve ark., 2010) bulunurken, mevcut çalışma bulgularını destekleyen çalışmalarda bulunmaktadır (Archer ve ark., 1997; Archer ve Bergh, 2000). Türkiye şartlarında hem GCAA hem de YYO oranı yönünden çalışmada ele alınan diğer ırklara göre daha iyi performans gösteren Simental ırkının tercih edilmesi işletmecilik açısından rasyonel karar olacaktır.

Diğer taraftan yapılan besicilikte performans göstergeleri kadar diğer önemli bir konu da karkas verimidir. Çünkü değerli etler (bonfile, kontrfile, antrikot vs.) yanında kesilen hayvanlardan elde edilen karkaslara ait sıcak/soğuk karkasların ağırlık ve oranları da besi sonunda elde edilecek gelir ve karlılıkları doğrudan etkilemektedir.

Karkas verimine ait veriler incelendiğinde, SIKA yönünden en yüksek ağırlıklar Simental (327 kg), İsviçre esmeri (288 kg) ve Kültür Melezi (280 kg) ırklarında

bulunmuştur. SIKAR yönünden en yüksek oranlar Kültür Melezi (%58.6), Simental (%57.7) ve Şarole (%57.4) ırkları ön plana çıkmaktadır. Literatürde, yapılan mevcut çalışmadan SIKA bakımından farklı sonuçların elde edildiği çalışmalar (Litwińczuk ve ark., 2014; Wheeler ve ark. 2005) olsa da, mevcut çalışmada olduğu gibi Simental ve Simental melezi ırkların SIKA bakımından diğer sığır ırklardan daha yüksek olduğunun bildirildiği çalışmalar bulunmaktadır (Barton ve ark., 2006; Holló ve ark., 2012; Mandell ve ark., 1997; Wang ve ark., 2021).

SOKA yönünden en yüksek ağırlıklar Limuzin (363.7 kg) ve Simental (320.8 kg) ırklarında bulunmuştur. Mevcut çalışmada olduğu gibi Simental ve Simantal melezlerinin SOKA ve SOKAR bakımından diğer ırklardan üstün olduğunun tespit edildiği çalışmalar bulunmaktadır (Dannenberger ve ark., 2006; Oliveira ve ark., 2011). Öte yandan diğer sığır ırklarının, mevcut çalışmada SOKA ve SOKAR bakımından yüksek değere sahip olan Simental ırkından daha yüksek SOKA ve SOKAR değerine sahip olduğunu bildiren çalışmalar bulunmaktadır (Martin ve ark., 1980; Wajda ve ark., 2006).

Sonuç olarak, tüm ticari faaliyetlerde olduğu gibi sığır besiciliğinde de en yüksek karlılık hedeflenmektedir. Yapılan çalışma ile Türkiye'de bölgesel olarak ırk tercihleri, besiye başlama yaşı ve besi süreleri gibi faktörlerin, karlılık üzerine etkisi olan besi performansı ve karkas randımanı göstergelerine yansımaları bütüncül bir yaklaşım olan meta analizi ile değerlendirilmiştir. Mevcut çalışma sonuçları, Türkiye şartlarında sığır besiciliği yapan/yapacak olan üreticiler ile politikacılara gelecekte sürdürülebilir ve daha karlı bir üretim yapılması için karar desteği sağlayacaktır.

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Yazar Katkıları

Fikir/Kavram: SS
Tasarım: SS
Denetleme/Danışmanlık: SS
Veri Toplama ve/veya İşleme: SS, EÇG
Analiz ve/veya Yorum: EÇG, GG
Kaynak Taraması: SS, MK
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Kaynaklar

Akgöz S, Ercan İ, Kan İ, 2004: Meta-analizi. *UÜTF*, 30 (2), 107-112.

Archer, JA, Arthur, PF, Herd, RM, Parnell, PF, Pitchford, WS, 1997: Optimum postweaning test for measurement of growth rate, feed intake, and feed efficiency in British breed cattle. *J Anim Sci*, 75(8), 2024-2032.

Archer, JA, Bergh L, 2000: Duration of performance tests for growth rate, feed intake and feed efficiency in four biological types of beef cattle. *Anim Prod Sci*, 65(1-2), 47-55.

Aydın B, Demirkol C, 2021: Çanakkale ili Ayvacık ilçesinde organik sığır besiciliği yapan işletmelerin ekonomik ve fonksiyonel analizi. *NKÜ Sos Bil Metinleri,* 2021 (1), 49-61.

Aygül H, Özkütük K, 2012: Malatya ili süt sığırcılığı ve sığır besiciliğinin yapısı. *Adana AVKAE Dergisi*, 2, 7-11.

Barton L, Rehak D, Teslık V, Bures D, Zahradkova R, 2006: Effect of breed on growth performance and carcass composition of Aberdeen Angus, Charolais, Hereford and Simmental bulls. *Czech J Anim Sci*, 51, (2), 47-53.

Baujat B, Mahé C, Pignon JP, Hill C, 2002: A graphical method for exploring heterogeneity in meta-analyses: application to a meta-analysis of 65 trials. *Stat Med*, 21(18), 2641-2652.

Borenstein M, Higgins JPT, 2013: Meta-analysis and subgroups. *Prev Sci*, 14 (2), 134-143.

Crowley, JJ, McGee, M, Kenny, DA, Crews JDH, Evans, RD, Berry, DP, 2010: Phenotypic and genetic parameters for different measures of feed efficiency in different breeds of Irish performance-tested beef bulls. *J Anim Sci*, 88(3), 885-894.

Dannenberger, D, Nuernberg, K, Nuernberg, G, Ender, K, 2006: Carcass-and meat quality of pasture vs concentrate fed German Simmental and German Holstein bulls. *Arch Anim Breed*, 49(4), 315-328.

Duru S, Sak H, 2017: Türkiye 'de besiye alınan simmental, aberdeen angus, hereford, limousin ve charolais ırkı sığırların besi performansı ve karkas özellikleri. *TURJAF*, 5 (11), 1383-1388.

Gözener B, Sayılı M, 2015: Tokat ili Turhal ilçesinde sığır besiciliğinde üretim maliyeti ve canlı ağırlık artışına etki eden faktörler. *J Agric Sci*, 21 (2), 288-299.

Hedges LV, Tripton E, 2010: Meta-analysis. Handbook of Behavioral Medicine: Methods and Applications. Springer, New York.

Holló G, Nuernberg K, Somogyi T, Anton I, Holló I, 2012: Comparison of fattening performance and slaughter value of local Hungarian cattle breeds to international breeds. *Arch Anim Breed*, 55(1), 1-12.

Jakubec V, Schlote W, Riha J, Majzlik I, 2003: Comparision of growth traits of eight beef cattle breeds in the Czech Republic. *Arch Anim Breed*, 46 (2), 143-153.

Kızıl SH, Aydoğan M, 2014: Evaluation of major cattle breeds in Turkey for slaughter and carcass traits using manova and multidimensional scaling technique. *Erciyes Üniv Vet Fak Derg*, 11 (1), 15-22.

Laborde FL, Mandell IB, Tosh JJ, Wilton JW, Buchanan-Smith JG, 2001: Breed effects on growth performance, carcass characteristics, fatty acid composition, and palatability attributes in finishing steers. *J Anim Sci*, 79(2), 355-365.

Litwińczuk Z, Żółkiewski P, Florek M, Chabuz W, Domaradzki P, 2014: Semi-intensive fattening suitability and slaughter value of young bulls of three Polish native breeds in comparison with Polish Holstein-Friesian and Simmental. *Ann Anim Sci*, 14(2), 453-460.

Mandell IB, Gullett EA, Wilton JW, Kemp RA, Allen OB, 1997: Effects of gender and breed on carcass traits, chemical composition, and palatability attributes in Hereford and Simmental bulls and steers. *Livest Prod Sci*, 49(3), 235-248.

Martin EL, Anderson DC, O'Mary CC, 1980: Carcass traits of and preweaning creep feeding effects on steers sired by Angus, Holstein, Simmental and Chianina bulls. *J Anim Sci*, 50(1), 62-66.

Oliveira IMD, Paulino PVR, Valadares Filho SDC, Detmann E, Paulino MF, Gomide LADM, Couto VRM, 2011: Carcass traits of Nellore, F1 Simmental× Nellore and F1 Angus× Nellore steers fed at maintenance or ad libitum with two concentrate levels in the diet. *Rev Bras de Zootec*, 40, 2938-2946.

Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Moher D, 2021: The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Int Surg J*, *88*, 105906.

Sağsöz Y, Çoban Ö, Laçin E, Sabuncuoğlu N, Yıldız A, 2005: Esmer ve şarole x esmer danaların besi performansı ve karkas özellikleri. *Atatürk Üniv Ziraat Fak Derg, 36*(2), 163-169.

Sakarya E, Günlü A, 1996: Limuzin x Jersey (F1) melezi ve Holştayn ırkı tosunlarda optimal besi süresinin tespiti üzerine bir araştırma. *Ankara Üniv Vet Fak Derg,* 43(1): 113-120.

Sever E, İğdeli A, Han V, 2017: Aksaray ili sığır işletmelerinin sosyo-ekonomik analizi. *J VetBio Sci Tech*, 2 (3), 1-11.

Şahin A, Miran B, Yildirim I, Önenç A, Alcicek A, 2009: Fattening costs of beef breeds reared under controlled conditions and the determination of optimum fattening period. *Turkish J Vet Anim Sci*, 33 (6), 485-492.

Tarım ve Orman Bakanlığı Hayvancılık Genel Müdürlüğü (HAYGEM). Hayvancılık İstatistikleri.https://www.tarimorman.gov.tr/sgb/Belgeler/SagMenuVeriler/HAYGEM.pdf. Erişim Tarihi: 15.03.2023

Tuncer Ş, 1984: Türkiye'de sığır besiciliğinin temel ilkeleri. *EJVS*, (1), 61-76.

Wajda S, Daszkiewicz T, Januškevičienė G, Dailidavičienė J, 2006): Fattening results and carcass quality of young bulls produced by mating Polish Black-and-White cows to Charolaise and Simental sires. *Vet ir Zootech*, 33, 84-89.

Wang Y, Wang Z, Hu R, Peng Q, Xue B, Wang L, 2021: Comparison of carcass characteristics and meat quality between Simmental crossbred cattle, cattle-yaks and Xuanhan yellow cattle. *J Sci Food Agric*, 101(9), 3927-3932.

Wheeler TL, Cundiff LV, Shackelford SD, Koohmaraie M, 2005: Characterization of biological types of cattle (cycle VII): Carcass, yield, and longissimus palatability traits. *J Anim Sci*, 83, 196-207.

HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Investigation of Metapodium and Acropodium Bones in Siirt-Colored Mohair Goat (*Capra hircus*) by 3D Modeling

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Abstract: Studies on Artiodactyla species benefit from the use of metapodial bones. Being one of the bones that completed its development early and being well preserved because it is not affected by environmental conditions, it can be unearthed as a single piece in archaeological excavations. In recent years, the disadvantages of 2D images have contributed to developing 3D modeling methods in medicine. Adult (1-3 years old), 10 male and 10 female Siirt-colored Mohair goat metacarpus and forelimb digit bones (phalanx proximalis, phalanx media and phalanx distalis) were used in our study. Bones were scanned with a computed tomography device. The resulting images were saved in DICOM format and 3D models were created using a special software. 14 osteometric measurements were taken from the metacarpus and 12 from the digit bones. The results were evaluated statistically. As a result of the statistical evaluation, in the metacarpus; Smallest Breadth of diaphysis (Sd), Breadth of distal (Bd), Medio-lateral width of condylus medialis width of condylus medialis (WCM), Depth of proximal (Dp), Antero-posterior diameter of external trochlea of condylus medialis (Dem) and Antero-posterior diameter of internal trochlea of condylus medialis (Dim) parameters were found to be statistically significant between males and females ($P<0.01$). For phalanx media, GLpe and Bp measurement parameters were statistically significant between genders ($P<0.05$). The data obtained from the study will contribute to anatomy education, the classification of bones obtained in zoo-archaeological excavations, and the determined anatomical features that will contribute to surgical approaches.

Keywords: *Acropodium, Metapodium, Siirt-colored Mohair goat (Capra hircus), 3D modelling.*

Siirt Renkli Tiftik Keçisinde (*Capra hircus*) Metapodium ve Acropodium Kemiklerinin 3D Modelleme ile İncelenmesi

Özet: Artiodactyla türlerine ilişkin çalışmalarda metapodial kemikler önemlidir. Gelişimini erken tamamlayan kemiklerden biri olması ve çevre koşullarından etkilenmemesi nedeniyle iyi korunmuş olması, arkeolojik kazılarda tek parça olarak ortaya çıkarılmasına olanak sağlamaktadır. Son yıllarda 2 boyutlu görüntülerin dezavantajları tıpta 3 boyutlu modelleme yöntemlerinin gelişmesine katkı sağlamıştır. Çalışmamızda yetişkin (1-3 yaş), 10 erkek ve 10 dişi Siirt renkli tiftik keçisinin metakarpusu ve ön ayak parmak kemikleri (phalanx proximalis, falanx media ve falanx distalis) kullanıldı. Kemikler bilgisayarlı tomografi cihazıyla tarandı. Ortaya çıkan görüntüler DICOM formatında kaydedildi ve özel bir yazılım kullanılarak 3 boyutlu modeller oluşturuldu. Metacarpus'tan 14, parmak kemiklerinden 12 osteometrik ölçüm alındı. Sonuçlar istatistiksel olarak değerlendirildi. İstatistiksel değerlendirme sonucunda metakarpusta; En küçük diyafiz genişliği (Sd), distal genişliği (Bd), condylus medialis medialis medialis genişliği (WCM), proksimal derinliği (Dp), condylus medialis eksternal troklea ön-arka çapı (Dem) ve Condylus medialis internal troklea ön-arka çapı (Dim) parametreleri dişiler ve erkekler arasında istatistiksel olarak anlamlı bulundu ($P<0.01$). Falanks ortamı için GLpe ve Bp ölçüm parametreleri cinsiyetler arasında istatistiksel olarak anlamlıydı ($P<0.05$). Çalışma sonucunda elde edilen verilerin anatomi eğitimine katkı sağlayacağı, hayvanat bahçesi-arkeolojik kazılarda elde edilen kemiklerin sınıflandırılması ve belirlenen anatomik özelliklerin cerrahi yaklaşımlara katkı sağlayacağı düşünülmektedir.

Anahtar Kelimeler: *Akropodyum, Metapodyum, Siirt renkli tiftik keçisi (Capra hircus), 3 boyutlu modelleme.*

Introduction

Bone remains obtained during archaeological excavations can provide information about the conditions of the day. The importance of these bones in terms of animal morphology is indisputable. Since metapodial bones are among the bones that finish developing early in life, they are significant in studies on Artiodactyla species. In addition, since it is well preserved and not affected by environmental conditions, it was generally revealed as a single piece during archaeological excavations (Bartosiewicz, 1985; Berteaux and Guintard 1995). For this reason, metapodium bones were examined in many excavations (Ince et al., 2018; Pazvant et al., 2015). The study of metapodial bones contributes to elucidating the history of sheep (Lamelland, 2002; Onar et al., 2008) and goat (Ince et al., 2018; Zeder 2001) domestication and estimating body conditions at that time (Guintard and Lallemand 2003; Lamelland, 2002). A study stating that the metapodium of the goat is shorter and broader than that of the sheep (Boessneck, 1969) supports the information that metapodial bones were used to distinguish the bones of sheep and goats in the Neolithic period (Rowley-Conwy, 1998).

Ruminants possess two fully developed digits, with three phalanges on each toe. Phalanx proximalis, phalanx medium, and phalanx distalis are the names of the three bones that comprise the digits (Bahadır and Yıldız 2016; Konig and Liebich 2020). The phalanx proximalis is the longest of them (Al-Sharoot, 2013). The length of the phalanx media is nearly half that of the phalanx proximalis. The nail bone, or phalanx distalis, is shaped differently from the bones of the other two fingers. In terms of surgery, it is crucial to understand the specific architecture of the metapodium and acropodium areas for the bone and the flexor and extensor muscles related to it (Demircioglu et al., 2021).

Using medical imaging equipment, two-dimensional (2D) pictures of significant bodily structures can be acquired. Various software can be used to study, measure, or divide images of the necessary anatomical structure (Demircioglu and Gezer 2020; Freitas et al., 2011; Ozkadif and Eken 2015; Sergovich et al., 2010; Yılmaz and Demircioglu 2021). The significance of 3D investigations is growing as a result of the drawbacks of 2D traditional imaging techniques as reduction, superposition, and magnification (Allowen et al., 2016; Aydogdu et al., 2021; Mirjana et al., 2014). Recently, one of the most popular approaches in medicine has been the use of three-dimensional modeling (D'Urso et al., 1999). Clinical instances, pathological cases, forensic medicine cases, and anthropological research all use reconstructive images (Verhoff et al., 2007). Furthermore, the utilization of computed tomography images in 3D modeling has gained popularity as a teaching tool for anatomy (Kong et al., 2016).

There are studies on the morphology and morphometry of metapodium bones from various sheep breeds, including Shetland sheep (Davis, 1996), Bardhoka (Gundemir et al., 2020), Hemsin (Gurbuz et al., 2018), Tuj and Morkaraman (Demiraslan et al., 2015), and Hamdani (Guzel et al., 2022). Pazvant et al. (2015) and Onar et al. (2008) also osteometrically examined sheep and goat metapodiums obtained from excavations in Istanbul and the Eastern Anatolia Region. Furthermore, investigations using 3D modeling were done on gazelles' acropodium bones (Demircioglu et al., 2021).

It is known that the majority of colored Mohair goats have been bred in Siirt, Batman and Sırnak provinces for a long time in Turkey (Gunes and Evrim 1993; Ilgaz and Sevinc 1982). These Mohair goats are crucial in weaving bags, vests, gloves, caps, socks and various ornaments offered to the market for touristic purposes, especially in weaving the famous Siirt blanket in the Siirt region. Siirt-colored Mohair goats are small in build. The Mohair covering them is thin, curved and shiny, black, white, gray, light red, brown, buff and yellow. The whole body is covered with Mohair except for the face and legs. Goats have a small, graceful head, bright and lively eyes. Although weak in females, the state of being horned is a general racial trait. The horns mostly follow a vertical course first and then laterally (Yerturk and Odabasıoglu 2007).

The aim of the study is to determine the morphometric characteristics of the metacarpus and forelimb digit bones of Siirt-colored Mohair goats by 3D modeling method, taking into account the gender difference. It is foreseen that the data obtained as a result of the study, the differences in terms of sex in Siirt-colored Mohair goats, and the measurements taken from the anatomically determined points will be used clinically and taxonomically.

Material and Methods

Adult (1-3 years old), 10 male and 10 female Siirt-colored Mohair goat metacarpus and forelimb digit bones (phalanx proximalis, phalanx media and phalanx distalis) were used in our study. The bones were obtained from animals slaughtered in the slaughterhouse of Siirt province. Following the maceration procedure, 12 measurements from the digit bones and 14 measurements from the metacarpus were obtained. Measurement points were determined with guidance from Guintard (1998), Guintard and Lallemand (2003) and von Den Driesch (1976). Measuring points are shown in Figures 1,2,3 and 4. The nomenclature was made in accordance with Nomina Anatomica Veterineria (2017). For three-dimensional measurements, a computerized tomography device in the department of radiology at Siirt University, Faculty of Medicine was used. A 64-detector MDCT scanner was used to scan the bones at 80 kV, 200 MA, 639 mGY, and 0.625 mm slice thickness. CT scans were recorded in the DICOM format according to the scan dose and protocol. Reconstructions were created with the help of the 3d slicer (5.02) software program. A segmentation technique was used on the CT images taken. The following osteometric measurements were made on the created 3D models. Statistical evaluation was made with the SPSS 22.0 program from the measurement results. The data from males and females were compared using an independent sample t-test.

Measurements points

Metacarpus (Figure 1)

GL: The greatest Length

Bp: The proximal breadth

Be: The metaphysis's maximum depth

De: Maximum metaphysis width

Sd: Smallest Diaphysis Breadth

Dp: Proximal Depth

BD: Distal breadth

Dd: Distal portion's depth

DIL: Antero-posterior diameter of internal trochlea of condylus lateralis

DEL: Antero-posterior diameter of external trochlea of condylus lateralis

DIM: Antero-posterior diameter of internal trochlea of condylus medialis

DEM: Antero-posterior diameter of external trochlea of condylus medialis

WCL: Medio-lateral width of condylus lateralis

WCM: Medio-lateral width of condylus medialis width of condylus medialis

Phalanx Proximalis (Figure 2)

A1. GLpe: Greatest abaxial half length (GLpe)

A2. Bp: The proximal end's breadth (Bp)

A3. SD: Diaphysis's smallest width (SD)

A4. Bd: The distal end's breadth (Bd)

Phalanx Media (Figure 3)

A5. GLpe: Greatest abaxial half length

A6. Bp: The proximal end's breadth

A7. SD: Diaphysis's smallest width

A8. Bd: The distal end's breadth

A9. GL: Greatest length (in dorsal direction)

Phalanx Distalis (Figure 4)

A10. DLS: The sole's longest diagonal length

A11. Ld: The dorsal surface's length

A12. MBS: Middle width of the sole

With the ethics committee report numbered 2023/03/24, the Siirt University Experimental Animals Application and Research Center approved the procedures used in our investigation.

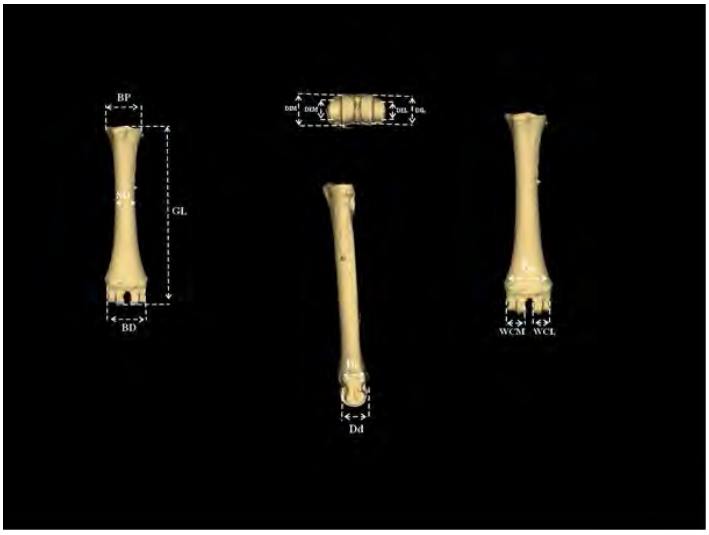

Figure 1: Osteometric measuring sites on the Siirt-colored Mohair goat's metacarpus bone.

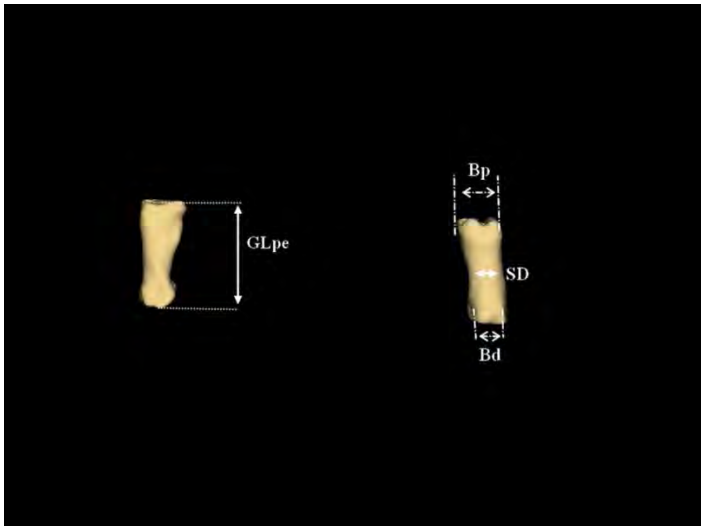

Figure 2: Osteometric measuring locations of the Siirt-colored Mohair goat's phalanx proximalis bone.

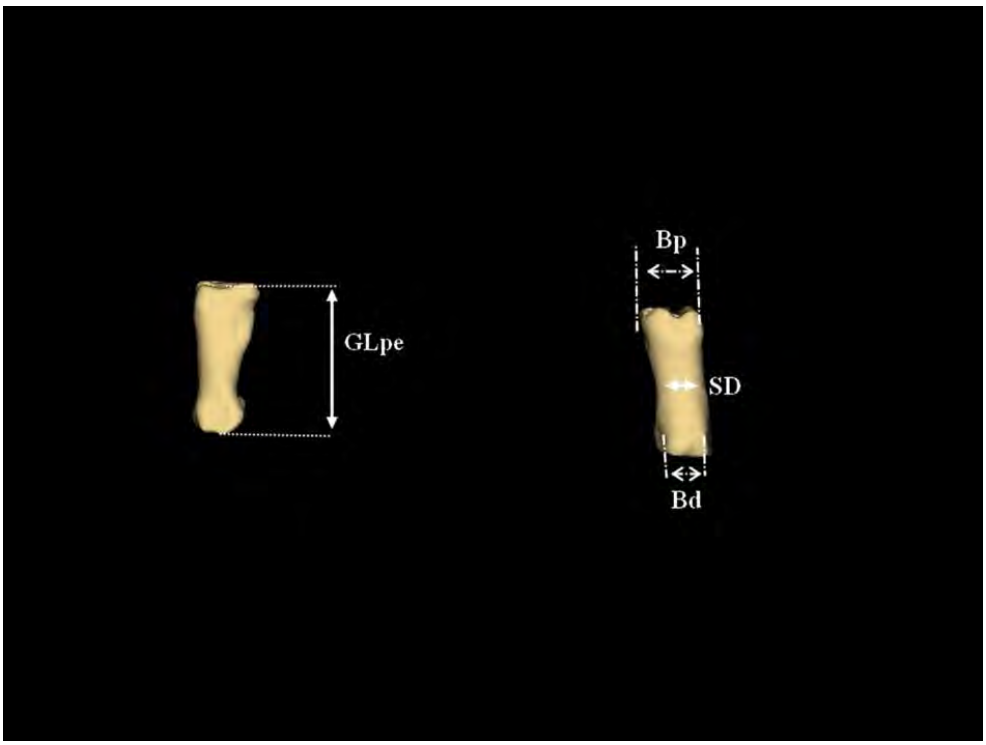

Figure 3: Osteometric measuring locations of the Siirt-colored Mohair goat's phalanx media bone.

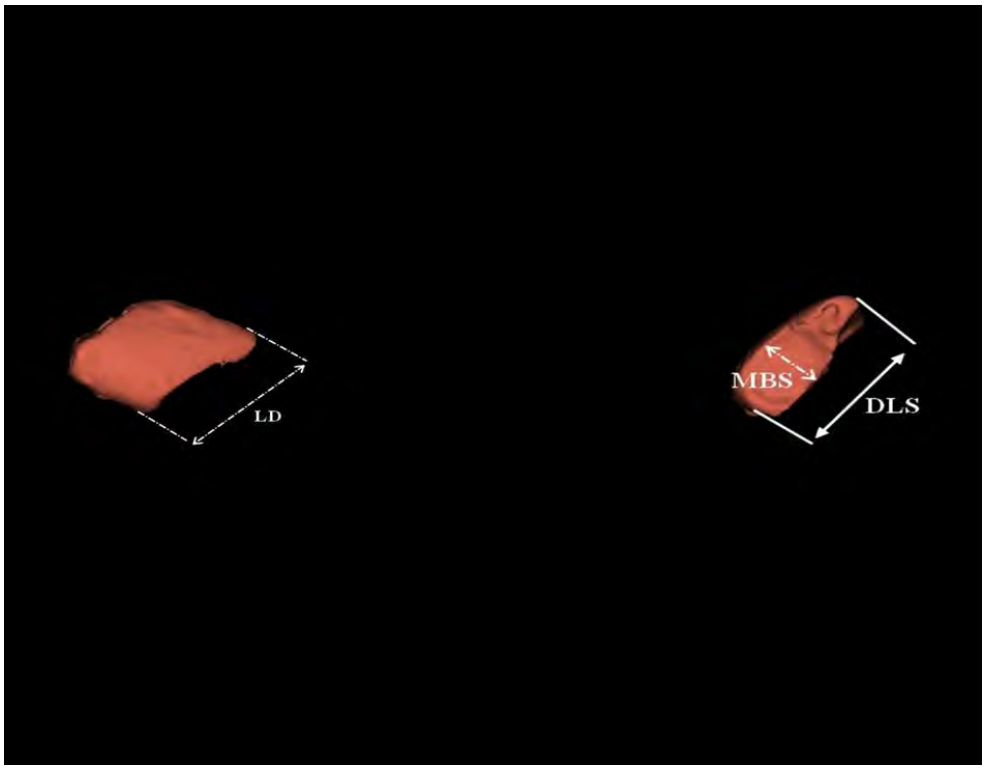

Figure 4: Osteometric measuring locations of the Siirt-colored Mohair goat's phalanx distalis bone.

Results

Tables 1 and 2 present the measurement results of the Siirt-colored Mohair goat's metacarpus and forelimb digit bones (phalanx proximalis, media, and distalis) from our investigation. Males were shown to have a greater metacarpus total length (GL) than females. The parameters measuring the Smallest Breadth of diaphysis (SD), Breadth of distal (Bd), Medio-lateral width of condylus medialis width of condylus medialis (WCM), Depth of proximal (Dp), Antero-posterior diameter of external trochlea of condylus medialis (DEM) and Antero-posterior diameter of internal trochlea of condylus medialis (DIM) were found to be statistically significant ($P<0.01$) when comparing males and females.

Table 1. Osteometric measurement results of metacarpus bones in Siirt-colored Mohair goats.

	Group	N	Mean	Std. Error of Mean	P
GL	Male	10	126.82	1.93	0.995
	Female	10	121.96	2.43	
Sd	Male	10	26.89	2.13	0.001
	Female	10	20.64	0.58	
Bp	Male	10	24.08	1.10	0.821
	Female	10	18.80	1.94	
BD	Male	10	21.04	2.45	0.002
	Female	10	13.85	0.25	
Be	Male	10	26.06	0.75	0.046
	Female	10	27.87	0.47	
WCM	Male	10	28.97	2.62	0.003
	Female	10	22.20	0.47	
WCL	Male	10	12.04	0.33	0.014
	Female	10	12.16	0.19	
Dp	Male	10	15.49	1.18	0.005
	Female	10	12.59	0.27	
DEM	Male	10	12.00	0.19	0.006
	Female	10	11.90	0.45	
DIM	Male	10	14.66	0.88	0.001
	Female	10	12.21	0.38	
DEL	Male	10	12.02	0.39	0.416
	Female	10	11.41	0.36	
DIL	Male	10	16.85	0.31	0.412
	Female	10	16.76	0.46	
Dd	Male	10	16.93	1.10	0.015
	Female	10	14.21	0.38	
De	Male	10	17.58	0.39	0.940
	Female	10	17.08	0.50	

Table 2. Osteometric measurement results of digit bones (phalanx proximalis, phalanx media and phalanx distalis) in Siirt-colored Mohair goats.

	Group	N	Mean	Std. Error of Mean	P
A1	Male	10	38.04	0.37	0.407
	Female	10	35.18	0.27	
A2	Male	10	17.31	0.42	0.609
	Female	10	15.57	0.26	
A3	Male	10	15.65	0.19	0.851
	Female	10	14.11	0.24	
A4	Male	10	16.12	0.19	0.009
	Female	10	14.33	0.11	
A5	Male	10	21.70	0.27	0.013
	Female	10	18.75	0.18	
A6	Male	10	14.37	0.13	0.024
	Female	10	13.17	0.24	
A7	Male	10	12.78	0.34	0.267
	Female	10	11.00	0.24	
A8	Male	10	20.55	0.24	0.824
	Female	10	19.19	0.19	
A9	Male	10	11.99	0.20	0.616
	Female	10	11.49	0.24	
A10	Male	10	62.15	0.36	0.534
	Female	10	58.31	0.41	
A11	Male	10	51.60	0.30	0.460
	Female	10	48.99	0.25	
A12	Male	10	38.55	0.34	0.556
	Female	10	34.58	0.23	

There was a significant difference between the genders in the maximum depth of the metaphysis (Be) and the depth of the distal (Dd) measuring sites ($P<0.05$). When the results of measurements of the phalanxes were examined, it was seen that the A1 and A5 measurement parameters were larger in males than in females. Considering the A4 measurement

point parameter, it was determined to be statistically significant (P<0.01). A5 and A6 measurement parameters were found to be statistically significant (P<0.05).

Discussion and Conclusion

In connection with the importance of mohair, although some selection studies have been carried out, especially in Ankara Mohair goats, conscious selection and breeding studies have not been carried out in colored Mohair goats (Yerturk and Odabasıoglu 2007). Morphological and morphometric characteristics of Siirt-colored Mohair goats are uncertain due to a lack of studies. While the Siirt-colored Mohair goat constitutes the majority of the colored Mohair goats raised in Turkey, it has great economic importance. Metapodium and acropodium bones in different species and races were evaluated osteometrically. However, such a study was not found in the Siirt-colored Mohair goat.

The aim of the study was to determine the morphometric characteristics of the metacarpus and forelimb digit bones of the Siirt-colored Mohair goat by performing osteometric analyses with 3D modeling and to reveal the differences between the sexes. The results obtained will help in the taxonomy of the bones obtained as a result of zoo-archeologic excavations.

The GL values in sheep and goat metacarpus unearthed in the Yenikapı metro and Marmaray excavations were 124.91±11.04mm and 115.52±9.44mm, respectively (Pazvant et al., 2015), 122.72±7.80mm in adult sheep, and 100.96±9.70mm in adult goats (Onar et al., 2008). In the study conducted in the Bardhoka sheep breed, this parameter was reported as 148.48±8.05mm in males and 139.19±6.61mm in females (Gundemir et al., 2020). In our study, the GL value was determined as 126.82±1.93mm in male goats and 121.96±2.43mm in females, and this parameter was found to be higher in male animals than in female animals.

In our study, the Sd parameter was determined as 26.89±2.13mm in male goats and 20.64±0.58mm in female goats, similar to Morkaraman sheep (Demiraslan et al., 2015), statistical differences were observed between male and female goats (P<0.01). The same parameter was reported as 13.29±0.79mm in males and 13.25±1.14mm in females in Hamdani sheep (Guzel et al., 2022). Gurbuz et al. (2018) reported this value as 15.10±0.34mm in Hemsin rams, while it was reported as 14.75±0.79mm in females and 13.64±0.50mm in males in Morkaraman sheep (Demiraslan et al., 2015).

Demiraslan et al. (2015) reported the BD value as 28.51±1.13mm, 27.89±1.37mm in Tuj sheep breeds in males and females, and 29.42±0.60mm and 27.47±3.55mm in Morkaraman sheep in their study in Tuj and Morkaraman sheep. While the exact value was reported as 27.22±0.86mm in females and 30.49±1.19mm in males in the Bardhoka sheep breed (Gundemir et al., 2020), it was determined as 25.12±1.04mm and 26.03±1.26mm in adult sheep and goats, respectively (Onar et al., 2008). In our study, this value was determined as 21.04±2.45mm in male goats and 13.85±0.25mm in females, and a statistically significant difference was observed between the sexes (P<0.01).

The WCM value was 12.66±1.21mm and 13.44±0.97mm in sheep and goat metacarpus unearthed in Yenikapı metro and Marmaray excavations (Pazvant et al., 2015). This parameter was determined as 12.06±0.83mm in males and 11.69±1.33mm in females in the Hamdani sheep breed (Guzel et al., 2022). In the present study, a significant difference was observed between female and male goats in terms of WCM value (P<0.01). While Onar et al. (2008) reported the Dp value as 16.75±0.87mm and 16.03±1.22mm in adult sheep and goats, respectively, this parameter was determined by Gundemir et al. (2020) as 19.5±1.23mm in females and 21.1±1.46mm in males in Bardhoka sheep. In our study, the Dp value was measured as 15.49±1.18mm in male goats and 12.59±0.27mm in female goats.

In the presented study, another parameter for which statistical difference was determined between the genders is the DEM parameter (P<0.01). While this parameter was reported as 13.50±0.36mm in Morkaraman rams and 12.99±0.57mm in sheep, in the same study it was determined as 13.139±0.73mm in Tuj rams and 13.14±0.55mm in sheep (Demiraslan et al., 2015). It has been reported to be 12.33±0.74mm in Hemsin rams (Gurbuz et al., 2018).

DIM parameter Pazvant et al. (2015) determined it to be 14.24±1.29mm and 14.75±1.40mm in sheep and goats, respectively, while the same parameter was reported as 15.40±2.42mm in Hamdani rams and 14.33±1.42mm in sheep (Guzel et al., 2022). In our study, the DIM parameter was determined as 14.66±0.88mm in male goats and 12.21±0.38mm in female goats. In addition, statistically significant differences were observed between the genders (P<0.01).

Be and Dd parameters, respectively, were reported as 27.09±0.73mm and 11.21±0.26mm in Hemshin rams (Gurbuz et al., 2018), 26.21±4.30mm and 12.30±2.23mm in Hamdani rams and 25.82±2.40mm and 11.27±1.41mm in Hamdani ewes (Guzel et al., 2022). Demiraslan et al. (2015) reported these two values as 31.36±1.14mm and 18.47±0.67mm in Morkaraman rams, 29.78±1.26mm and 16.54±1.52mm in sheep, respectively. In the same study, the values were reported as 31.02±1.51mm and 17.79±0.74mm in Tuj rams, 28.39±3.31mm and 17.37±3.31mm in sheep, respectively. In our study, as in Morkaraman sheep, a significant difference was determined between the sexes regarding Be and Dd parameters (P<0.05).

According to a study by Gundemir et al. (2020), the forelimbs had a greater GLpe measuring parameter of the phalanx proximalis than the hindlimbs. In black Bengal goats, Siddiqui et al. (2008) observed this value to be 1.88±0.03cm in the phalanx media of the forelimbs, and in gazelles, Demircioglu et al. (2021) reported it to be 1.95±5.59cm. According to sexual dimorphism, the GLpe parameter in the study on gazelles was shown to be significant for all extremities, with the exception of the left hind extremity phalanx media (Demircioglu et al., 2021). In our study, it was determined that the GLpe parameter in phalanx proximalis was higher in male goats than in female goats. In addition, it

was observed that the GLpe parameter had a significant difference between genders for phalanx media (P<0.05).

According to Nourinezhad et al. (2012), there was a statistically significant variation in the breadth of the distal end (Bd) of the proximal phalanx in buffaloes. Furthermore, Demircioglu et al. (2021) found that there was a statistically significant difference in the Bd values of the left forefoot inner, right forefoot inner, right forefoot outer, and right hindfoot outer bones between male and female gazelles. Similar to this, our study revealed a significant difference (P<0.01) in the Bd value for the phalanx proximalis between the genders. In addition, in our study, it was determined that the Bd value for the phalanx proximalis bone was higher in male goats than in female goats.

The GL, SD, and Bp values of the phalanx media were shown to be statistically significant in investigations on domestic cattle and water buffaloes (Gundemir et al., 2020; Nourinezhad et al., 2012). According to Bp, SD measures, and index values, there was no statistically significant sexual dimorphism seen in gazelles (Demircioglu et al., 2021). However, in our study, sexual dimorphism was determined in the phalanx media bone in terms of the Bp parameter (P<0.05).

As a result of the study, it was seen that certain values of metacarpus and forelimb digit bones in Siirt-colored Mohair goats showed statistically significant differences according to gender. For this reason, due to the compact structure of metapodium and acropodium bones, it is clear that they will make a great contribution to taxonomy if they are obtained in zoo-archaeological excavations. This study, in which the morphometric characteristics of the metacarpus and digit bones of the Siirt-colored Mohair goat, which has different phenotypic properties, are determined by 3D modeling, will help to classify the bones obtained in the excavations. In addition, we think that it will be important to know the morphometric structure of the region in surgical approaches to the metapodial and digit bones.

Conflict of Interest

The authors stated that they did not have anyreal, potential or perceived conflict of interest.

Ethical Approval

The procedures applied in our study were approved by the Siirt University Experimental Animals Application and Research Center with the ethics committee report numbered 2023/03/24.

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Similarity Rate

We declare that the similarity rate of the article is 14% as stated in the report uploaded to the system.

Author Contributions

Motivation / Concept: Fİ, BCG
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Analysis and/or Interpretation: BCG
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Critical Review: Fİ, BCG

References

Al-Sharoot HA, 2013: Anatomical study of the digits of fore limbs in goat. *QJVMS,* 12, 28-35.

Aydogdu S, Eken E, Koçak M, 2021: Sexual dimorphism in the sheep corpus callosum using 3 tesla MRI. *EJVRS,* 37(4), 225 - 234.

Bahadır A, Yıldız H, 2016: Veteriner Anatomi: Hareket Sistemi & İç Organlar. 7th ed., Ezgi Bookselling, Bursa, Turkey.

Bartosiewicz L, 1985: Interrelationships in the formation of cattle long bones. *Zool Anz Das,* 3, 253– 262.

Berteaux D, Guintard C, 1995: Osteometric study of the metapodials of Amsterdam Island feral cattle. *Acta Therio,* 40, 97– 110.

Boessneck J, (1969). Osteological differences between sheep (Ovis aries Linne) and goat (Capra hircus Linne) In: Brothwell D, Higgs E (Ed), 331-358, Science in Archaeology, London.

Courtenay LA, Maté-González MÁ, Aramendi J, Yravedra J, González-Aguilera D, Domínguez-Rodrigo M, 2018: Testing accuracy in 2D and 3D geometric morphometric methods for cut mark identification and classification. *Peer J,* 5(6), e5133.

Davis SJ, 1996: Measurements of a group of adult female Shetland sheep skeletons from a single flock: a baseline for zooarchaeologists. *J Archaeol Sci*, 23(4), 593-612.

Demiraslan Y, Gurbuz I, Aslan K, Akbulut Y, 2015: The stereological and morphometrical analysis of metapodium in Tuj and Morkaraman sheep. *ARC J Anim Vet Sci,* 1(1), 12–23.

Demircioglu I, Gezer IN, 2020: Threedimensional modelling of computed tomography images of limb bones in gazelles (Gazella subgutturosa). *Anat Histol Embryol,* 49, 695–707.

Demircioğlu İ, Koçyiğit A, Demiraslan Y, Yilmaz B, Gezer Ince N, Aydogdu S, Dayan MO, 2021: Digit Bones (Acropodium) of Gazella (Gazella subgutturosa); Three-Dimensional Modelling and Morphometry. *Pak Vet J,* 41(4), 481-486.

D'Urso PS, Barker TM, Earwaker WJ, Bruce LJ, Atkinson RL, Lanigan MW, Arvier JF, Effeney DJ, 1999: Stereolithographic biomodelling in cranio-maxillofacial surgery: a prospective trial. *J Maxillofac Surg,* 27(1), 30–37.

Evin A, Souter T, Hulme-Beaman A, Ameen C, Allen R, Viacava P, Larson G, Cucchi T, Dobney KM 2016: The use of close-range photogrammetry in zooarchaeology: Creating accurate 3D models of wolf crania to study dog domestication. *J Archaeol Sci Rep,* 9, 87-93.

Freitas EP, Noritomi PY, Silva JVL, 2011: Use of rapid prototyping and 3D reconstruction in veterinary medicine. InTech.

Guintard C, 1998: Osteometrie des metapodes de bovins. *Revue Med Vet*, 149, 751-770.

Guintard C, Lallemand M, 2003: Osteometric study of metapodial bones in sheep. *Ann Anat,* 185, 573– 583.

Gundemir O, Pazvant G, Jashari T, Dayan MO, 2020: Morphometric Study of Metapodium in Bardhoka Sheep. *J Agric Vet Sci*, 4, 30–38.

Gunes H, Evrim M, 1993: Turkiye ve Amerika Birlesik Devletleri orijinli Ankara keçisi hatları arasındaki birlestirmelerden elde

edilen çesitli genotip gruplarının onemli verim ozellikleri yonunden karsılastırılması. I. Tiftik verimi ve tiftik ozellikleri. *İstanbul Univ Vet Fak Derg,* 19(1), 83-99.

Gurbuz İ, Demiraslan Y, Kırbas G, Aslan K, 2018: Hemsin Koyunlarında Metapodium'ların Morfometrik ve Stereolojik İncelenmesi. *MAKÜ Sag Bil Enst Derg,* 6(1), 1–14.

Guzel BC, Koçyigit A, Demircioglu İ, Demiraslan Y, 2022: Investigating metacarpi of Hamdani sheep via different measurement and modelling methods: A methodological study. *Anat histol embryol,* 51(4), 484–491.

Ilgaz B, Sevinç A, 1982: Ankara keçilerinde kızgınlık, kızgınlık siklusu sureleri ve en uygun tohumlama zamanı. *Lalahan Zootekni Ars Enst Derg,* 22, 1-4, 61-69.

Ince NG, Pazvant G, Sarıtas O, Kahvecioglu KO, Ozturk M, Onar V, 2018: Osteometrical assessment of withers height and sex determination of byzantine cattle from metacarpals (The theodosiusharbour area, Istanbul). *MAA,* 18(1), 46-60.

Kong X, Nie L, Zhang H, Wang Z, Ye Q, Tang L, Li J, Huang W, 2016: Do Three-dimensional Visualization and Three-dimensional Printing Improve Hepatic Segment Anatomy Teaching? A Randomized Controlled Study. *J Surg Educ,* 73(2), 264–269.

Konig HE, Liebich HG, 2020: Veterinary anatomy of domestic animals: Textbook and colour atlas. Georg Thieme Verlag, New York, USA.

Lallemand M, 2002: Etude ostéométrique de métapodes de mouton (Ovis aries, L). PhD Thesis, Ecole Nationale Veterinaire de Nantes, Nantes.

Mirjana D, Concepción R, Patricia G, Inmaculada A, 2014: Morphometric sex estimation from 3D computed tomography os coxae model and its validation in skeletal remains. *Int J Legal Med,* 128, 879–888.

Nomina Anatomica Veterinaria, 2017: Nomina Anatomica Veterinaria. World Association of Veterinary Anatomist, New York.

Nourinezhad J, Mazaheri Y, Daneshi M, 2012: Morphometric study on digital bones in native Khuzestan Water Buffaloes (Bubalus bubalis). *Bulg J Vet Med,* 15, 228-35.

Onar V, Pazvant G, Belli O, 2008: Osteometric examination of metapodial bones in sheep (Ovis aries L.) and goat (Capra hircus L.) unearthed from the Upper Anzaf Castle in Eastern Anatolia. *Rev Med Vet,* 159, 150-158.

Ozkadif S, Eken E, 2015: Contribution of virtual anatomic models to medical education. *Ataturk Univ Vet Bilim,* 10, 46–54.

Pazvant G, Onar V, Alpak H, Gezer Ince N, Kahvecioglu KO, Armutak A, Kızıltan Z, 2015: Osteometric examination of metapodial bones in sheep (Ovis aries L.) and goat (Capra hircus L.) unearthed from the Yenikapı metro and Marmaray excavations in Istanbul. *Kafkas Univ Vet Fak Derg,* 21(2), 147–153.

Rowley-Conwy P, 1998: Improved separation of Neolithic metapodials of sheep (ovis) and goats (capra) from Arene Candide cave, Liguria, Italy. *J Archaeol Sci,* 25, 251-258.

Sergovich A, Johnson M, Wilson TD, 2010: Explorable three dimensional digital model of the female pelvis, pelvic contents, and perineum for anatomical education. *Anat Sci Educ,* 3, 127–133.

Verhoff MA, Ramsthaler F, Krähahn J, Deml U, Gille RJ, Grabherr S, Thali M, Kreutz K, 2008: Digital forensic osteology--possibilities in cooperation with the Virtopsy project. *Forensic Sci. Int.,* 174 (2-3), 152–156.

Von den Driesch A, 1976: A guide to the measurement of animal bones from archaeological sites. Peabody Museum of Archaelogy and Ethnology, Harvard University, Cambridge, Mass, USA.

Yerturk M, Odabasıoglu F, 2007: Dogu ve Guneydogu Anadolu Bolgesinde Yetistirilen Renkli Tiftik Keçilerinin Yarı Entansif Sartlarda Verim ozelliklerinin Arastırılması. *Van Vet J,* 18 (2): 45-50.

Yilmaz O, Demircioglu İ, 2021: Three-dimensional reconstruction and morphometric analysis of the mandible in Van cats: A computed tomography (CT) study. *Pol J Vet Sci,* 24(2), 261–270.

Zeder MA, 2001: A metrical analysis of a collection of modern goats (Capra hircus aegargus and C. H. Hircus) from Iran and Iraq: Implications for the study of caprine domestication. *J Archaeol Sci,* 28, 61-79.

HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Harpagophytum procumbens Ekstraktının Klinik Kullanımı

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Özet: Günümüzde tedavide kullanılan ilaçların bazılarının yan etkileri ve toksisiteleri nedeniyle fitoterapi ve doğal tedavilere yönelim artmıştır. Tıbbi ve aromatik bitkiler içerisinde yer alan *Harpagophytum procumbens* (HP), meyvesi dış görünümü nedeniyle Şeytan pençesi olarak da isimlendirilmiştir. Şeytan pençesi bitkisi genellikle Güney Afrika'nın Kalahari Çölü, Namibya, Botswana, Zambiya, Zimbabwe ve Mozambik'te yetişen otsu bir bitkidir. Geleneksel tıpta, şeytan pençesi uzun yıllardır infüzyon, kaynatma, tentür, toz ve özüt formlarında kullanılmaktadır. Bitkinin köklerinde bulunan ana kimyasal bileşikler; iridoid glikozitler, triterpenoitler (oleanolik ve ursolik asit), şekerler (tetrasakkarit, stakioz), aromatik asitler (kafeik, sinnamik ve klorojenik asitler), fitosteroller (beta-sitosterol) ve flavonoitlerdir (luteolin ve kemferol). Son yıllarda yapılan araştırmalarda harpagosid ve harpagid gibi biyoaktif bileşenlerin, antienflamatuar, analjezik ve antioksidan etkilerinin olduğu bildirilmiştir. HP ekstraktlarındaki antioksidan aktiviteden sorumlu bileşenler, serbest radikal süpürücüsü olarak bilinen flavonoidler, hidrojen donörleri ve oksijen radikal nötürleştiricileri olarak işlev gören bitki fenolleridir. Klinik olarak şeytan pençesi sedatif, ateş düşürücü, öksürük kesici, yara iyileştirici, ülser ve çıbanlar gibi deri lezyonları ve hazımsızlık tedavisinde ayrıca osteoartrit, romatoid artrit, tip 2 diyabet, obezite, kan hastalıkları, kanser, kardiyovasküler ve pulmoner hastalıklar gibi çeşitli hastalıkların tedavisinde kullanılmaktadır. Bu makalede HP'nin genel özellikleri ve klinik kullanımı mevcut literatürlerden yararlanılarak derlenmiştir.

Anahtar Kelimeler: *Analjezik, Antienflamatuar, Antioksidan, Şeytan pençesi.*

Clinical Usage of *Harpagophytum procumbens* Extract

Abstract: Due to the side effects and toxicities of some drugs used in treatment today, the orientation towards phytotherapy and natural therapies has increased. *Harpagophytum procumbens* (HP), which is among medicinal and aromatic plants, is also called Devil's Claw because of the external appearance of its fruit. Devil's claw is a herbaceous plant predominantly grown in South Africa's Kalahari Desert, Namibia, Botswana, Zambia, Zimbabwe, and Mozambique. In traditional medicine, Devil's claw has been used in the forms of infusions, decoctions, tinctures, powders, and extracts for many years. The main chemical compounds found in the plant's roots include iridoid glycosides, triterpenoids (oleanolic and ursolic acid), sugars (tetrasaccharide, stachyose), aromatic acids (caffeic, cinnamic, and chlorogenic acids), phytosterols (beta-sitosterol), and flavonoids (luteolin and chempherol). In recent years, it has been reported that bioactive components such as harpagoside and harpagid have anti-inflammatory, analgesic, and antioxidant effects. The features responsible for the antioxidant activity in HP extracts are flavonoids known as free radical scavengers, plant phenols that act as hydrogen donors, and oxygen radical neutralizers. Clinically, Devil's claw is used as a sedative, antipyretic, antitussive, wound healer in the treatment of skin lesions such as ulcers and abscesses, and dyspepsia, as well as in the treatment of various diseases such as osteoarthritis, rheumatoid arthritis, type 2 diabetes, obesity, blood diseases, cancer, cardiovascular and pulmonary diseases. This article reviews the general characteristics and clinical usage of HP using the available literature.

Keywords: *Analgesic, Antiinflammatory, Antioxidant, Devil's claw.*

Giriş

Kronik hastalıklarda sık kullanılan özellikle non steroidal antienflamatuar (NSAID) ilaçların kalp, böbrek ve karaciğer yetmezliği ile gastrointestinal lezyonlar gibi sık görülen yan etkilerinden dolayı araştırmacılar çeşitli doğal alternatiflere yönelmişlerdir (Bindu ve ark., 2020; Nyberg ve ark., 2020; Rotunno ve ark., 2018). Geleneksel tıpta enflamasyon süreçlerinin altında yatan temel mekanizmaları etkileyebilecek çok sayıda bitki bulunduğu bilinmektedir. *Harpagophytum procumbens* (HP), bu tür bitkiler arasında üzerinde en çok araştırma yapılan bitkilerden biri olma özelliğini taşımaktadır (Asadi-Samani ve ark., 2017).

Özellikle Güney Afrika'nın Kalahari Çölü başta olmak üzere Angola, Botsvana, Namibya gibi ülkelerde yaygın şekilde yetiştiği bilinen HP; şeytan pençesi, çeneli bitki, ağaç örümceği olarak da bilinmektedir. Bitkinin adı odunsu kollarının üzerinde bulunan dikensi çıkıntıya benzer görünümlerinden ileri gelmekte ve bitki büyük su depolayan borulu kökleri ile tanınmaktadır. Şeytan pençesi, Güney Afrika'da bulunan yerliler tarafından bazı hastalıkların tedavisinde uzun yıllardır kullanmaktadır. Bitkinin köklerine ait içeriklerin özellikle ağrı ve iltihap tedavisinde etkili olduğu yapılan çalışmalarla da kanıtlanmıştır (Khumalo ve ark., 2022). Şeytan pençesi köklerinin antienflamatuar etkisinin içerdiği iridoid glikozit ve harpagosidlere bağlı olduğu bildirilmiştir (Mncwangi ve ark., 2012).

Meyvesinin dış görüntüsü nedeniyle şeytan pençesi olarak adlandırılan HP'nin yumrulu köklerinin, hazımsızlık, kan hastalıkları, menstrual problemler, yara, ülserler, çıban, frengi, ateş, iştah kaybının tedavisi ve hatta harici kanserli büyümeler dahil diğer cilt lezyonlarının tedavisi için yaygın olarak kullanıldığı bilinmektedir. HP, infüzyon, kaynatma, tentür, toz ve özüt şeklinde farklı hastalıkların tedavileri için de kullanılır. Ayrıca Alzheimer, obezite, tip 2 diabet, kardiovaskuler ve pulmoner hastalıklar, artrit, romatizmal, bakteriyel, fungal ve viral hastalıklarda da kullanıldığı belirtilmiştir (Gxaba and Manganyi, 2022; Serrano ve ark., 2018).

***Harpagophytum procumbens* Kullanımının Tarihsel Süreci:** HP yumrularının yüzyıllardır Güney Afrika'daki yerli halk (San, Khoi, Bantu halkı) tarafından bitkinin sekonder yumrularının kaynatılarak veya tentür, toz, özüt şeklinde ilaç olarak kullanıldığı rapor edilmiştir (Van den Eynden ve ark., 1992; Watt ve Breyer-Brandwijk, 1962). Avrupalı bilim adamları tarafından HP ilk defa 1820 yılında bulunmuş ve 1900'lü yılların başından itibaren tedavi amacıyla resmi olarak kullanılmaya başlanmıştır (Stewart ve Cole, 2005).

HP, farmakolojik aktivite analizi ve klinik testleri 1960'lı yıllardan bu yana yapılan ve en çok araştırılan tıbbi bitkilerden biri olarak başı çekmektedir (Georgiev ve ark., 2012). 1960'lı yıllarda HP Avrupa'ya tanıtılarak Harpagophyti radix farmasötik adıyla ticareti yapılmıştır. Şeytan pençesi ürünleri Fransa ve Almanya'da bitkisel ilaç, İngiltere, Hollanda ve Uzak Doğu'da gıda takviyesi olarak ruhsatlandırılmıştır. Son yıllarda şeytan pençesi, aspirin, ibuprofen gibi NSAI ilaçlara ve ayrıca reçeteli NSAI ilaçlara (rofekoksib ve selekoksib) bir alternatif olarak kabul edilmektedir (Stewart ve Cole, 2005).

Farmakolojik İçeriği: HP'nin köklerinde bulunan esas kimyasal yapıları; iridoid glikozitler, triterpenoitler (oleanolik ve ursolik asit), şekerler (özellikle tetrasakkarit, stakioz), aromatik asitler (kafeik, sinnamik ve klorojenik asitler), fitosteroller (beta-sitosterol) ve flavonoitler (luteolin ve kemferol) oluşturmaktadır. HP bitkisinin ana bileşenlerini oluşturan iridoid glikozitlerin biyoaktif bileşik olarak en çok araştırılan ve standardizasyon amacıyla belirteç olarak kullanılanı harpagosiddir (Açıkgöz ve ark., 2020). Bunun dışında daha az oranda harpagit ve prokumbit de araştırılmıştır. Avrupa'da standart ürünlerde en az %1.2 harpagozit bulunması istenmektedir (Anauate ve ark., 2010). Yapılan bir çalışmada HP kök ekstresinin antienflamatuvar etkisinin harpagozit bileşeninden kaynaklandığı belirtilse de bu etki için harpagozitin tek başına yeterli olmadığı ve istenilen etkinin harpagozitin diğer bileşiklerle sinerjist etkileşiminden kaynaklandığı belirtilmiştir (Anauate ve ark., 2010).

Yapılan araştırmalar sonucunda bitkinin ana kimyasal bileşenlerinin ikincil yumrularda birincil yumrulara göre yaklaşık olarak iki kat daha fazla harpagosit içerdiği ifade edilmiştir. Bitkinin çiçek, sap ve olgun meyvelerinde harpagosid bulunmadığı belirtilmiştir. Yapraklarında ise tanımlanamayan bazı iridoid bileşiklerle birlikte harpagosid izlerine rastlanmıştır (Açıkgöz ve ark., 2020).

Farmakolojik Etkileri ve Klinik Kullanımı: Şeytan pençesi bitkisinin, geleneksel tıpta tarihsel olarak antienflamatuar, analjezik, antioksidan, antidiyabetik, antibakteriyel, antiviral, antifungal, antiprotozoal, kemopreventif, nöroprotektif, hepatoprotektif ve immunomodülatör olarak kullanıldığı belirtilmiştir (Atay ve ark., 2016; Grant ve ark., 2007; Serrano ve ark., 2018; Villasenor, 2007). Bu etkilerinden bazıları ile ilgili yapılan çalışmalar;

Antienflamatuar Etkisi: HP'nin antienflamatuar etkisini içerdiği harpagosidin siklooksijenaz-1 (COX-1) ve siklooksijenaz-2 (COX-2) enzimlerinin inhibisyonu ve proinflamatuar sitokinler ile nitrik oksit (NO) üretiminin regülasyonu ile gösterdiği belirtilmiştir (Mncwangi, 2012). Harpagozitin nükleer faktör-kappa B'yi inhibe ederek ve LPS kaynaklı iNOS ve COX-2 ekspresyonunun da inhibisyonunu sağlayarak antienflamatuvar etki gösterdiği tespit edilmiştir (Huang ve ark., 2006; Kaszkin ve ark, 2004). Bu mekanizmanın, COX-2, lökotrienler, tümör nekroz faktörü-alfa (TNF-α) ve interlökin-1 (IL-1) gibi enflamatuvar mediatörlerin inhibisyonu yoluyla olduğu da bildirilmiştir (Chrubasik ve ark., 2006). Ayrıca yapılan başka bir çalışmada HP'nin biyoaktif bileşenlerinden biri olan harpagosidin anti-inflamatuar özelliklere sahip olup olmadığı, IL-6 ve matriks parçalayıcı proteazlar gibi enflamatuar sitokinlerin/kemokinlerin üretimini baskılama potansiyelinin olup olmadığı osteoartrit modelinde araştırılmıştır. Bu araştırmada, harpagosidin, patolojik koşullar altında osteoartrit kondrositlerinde c-FOS/AP-1 aktivitesini baskılayarak ve aracılık ettiği enflamatuar uyaranları inhibe ederek önemli bir antienflamatuar etki gösterdiği tespit edilmiştir (Haseeb, 2017).

HP köklerinin (yaklaşık %1,5 harpagosid içeren) sulu ekstraktının, kolon dokusunda lipid peroksidasyonu ve enflamasyonu inhibe ettiği belirlenmiştir (Locatelli ve ark., 2017). HP kök ekstraktlarının içerdiği β karyofilen gibi uçucu bileşiklerin de aralarında bulunduğu birkaç bileşik, bitkilerin patojen ve mantar enfeksiyonlarına karşı bir savunma stratejisi olarak üretilmiştir (Gfeller ve ark., 2019). β-karyofilen ve öjenolün antienflamatuar etkiden sorumlu oldukları, β-karyofilenin hem nosiseptif hem de antienflamatuar yollarda yer alan CB2 reseptörünün bir agonisti olarak hareket ettiği gösterilmiştir (Chicca ve ark., 2014).

Analjezik Etkisi: Alternatif tıbba yönelimin en büyük nedenlerinden birisi analjezik ilaçların uzun süreli kullanımına bağlı gelişen tolerans ve yan etkiler olmuştur. Yapılan çalışmalarda şeytan pençesinin kök ekstraktının (50-800 mg/kg), farelerde deneysel oluşturulan ağrı uyaranlarına karşı önemli analjezik etkilerinin olduğu belirtilmiştir (Mahomed and Ojewole, 2005). Uchida ve ark. (2008) HP ekstraktının sıçanlarda formalin testinde önemli antinosiseptif etkiler gösterdiğini ve bu etkide opioiderjik sistemin rol oynadığını belirtmişlerdir. Sıçan omuriliğinde formalin enjeksiyonu sonucu artan nitrit/nitrat (NOx) seviyesinin HP ekstresi ile önemli derecede düştüğü de tespit edilmiştir.

Parenti ve ark. (2015) 800 mg/kg dozda HP ekstresinin intraperitoneal uygulanması ile sıçanlarda deneysel karragenan (İrlanda yosunu) kaynaklı oluşturulan ağrıda önemli derecede azalma gözlemlemişlerdir. Parenti ve ark. (2016) yaptıkları başka bir çalışmada ise sıçanlarda deneysel olarak oluşturulan nöropatik ağrıda, HP ekstresi ve morfin kombinasyonunun şiddetli ağrıya karşı sinerjistik bir şekilde analjezi oluşturduğunu belirlemişlerdir.

Lim ve ark. (2014) sıçanlarda nöropatik ağrı, plantar insizyon ile postoperatif ağrı oluşturdukları siyatik sinir ağrı modelinde, HP ve etanol ekstresi uygulamasından sonra elde edilen sonuçları değerlendirdiklerinde, sıçanlarda akut postoperatif ve kronik nöropatik ağrı durumunda potansiyel analjezik etki oluşturduğunu gözlemlemişlerdir.

Antioksidan Etkisi: Doğal kaynaklardan elde edilen antioksidanlar, artrit ve kanser gibi oksidasyona bağlı hastalıkların yüksek insidansı nedeniyle son yıllarda popülerlik kazanmıştır (Betancor ve ark., 2010). HP ekstraktlarındaki antioksidan aktiviteden sorumlu bileşenlerin, serbest radikal olarak bilinen flavonoidler, hidrojen donörleri ve oksijen radikal nötürleştiricileri olarak işlev gören bitki fenolleri olabileceği ifade edilmiştir (Sawa ve ark., 1999).

HP ekstraktlarının antioksidan ve antiinflamatuar etkilerinin harpagoside, gallik asit, kateşin, epikateşin, resveratrol, fenolik ve flavonoidlerden kaynaklandığı bildirilmiştir. Bu ekstraktın Candida albicans ve C. tropicalis gibi kolon iltihabı ile ilişkili olan bakteri ve mantar suşları üzerinde de etkili olduğu rapor edilmiştir (Iliev ve ark., 2012). Ayrıca, HP ekstraktı ile tedavi edilen kolonun proteomik analizinde, peroksiredoksin-2, glutatyon redüktaz, katalaz ve süperoksit dismutaz seviyelerinde artışa neden olduğu bildirilmiştir (Recinella ve ark., 2020).

Antidiyabetik Etkisi: HP yumrularının Güney Afrika'nın bazı topluluklarında uzun yıllardır tip 2 diyabet tedavisinde kullanıldığı belirtilmiştir. Diyete bağlı obez sıçanlarda glikoz intoleransını iyileştirmede antienflamatuar iridoid bileşik geninin önemli olduğu yapılan çalışmalarda gözlemlenmiştir (Guan ve ark., 2018; Mahomed and Ojewole, 2005).

HP ile Yapılan Diğer Deneysel Çalışmalar: Bitki kaynaklarından geliştirilip alternatif tedavide kullanılan binlerce formülasyon bulunmaktadır. Dünya Sağlık Örgütüne göre, gelişmekte olan ülkeler dahil olmak üzere, birçok hastalığın tedavisinde öncelikli tedavi seçeneği olarak geleneksel bitkisel ilaçlardan faydalanılmaktadır. Tedavide kullanılan bitkiler üzerine yapılan çalışmalar terapötik etkiler ve olası yan etkilerin belirlenmesi için büyük klinik öneme sahiptir (Cragg ve ark., 2013).

Üçüncü ve ark. (2015) hayvanlarda uygulanan deneysel romatoid artrit modelinde, hayvanlara 30 gün boyunca oral olarak HP ekstresi ile kombine halde glukozamin hidroklorür, bromelain ekstresi, kondroitin sülfat ve metilsülfonilmetanı uygulamışlardır. % 3 harpagozit içeren ekstrenin, bu kombinasyonla kıkırdak yıkımını önlediğini belirtmişlerdir. Yapılan analizler neticesinde malondialdehid, NO, 8-hidroksiguanin seviyelerini ve proenflamatuvar sitokin gen seviyelerini düşürerek oksidatif stres ve enflamasyonu baskıladıklarını bildirmişlerdir.

Haseeb ve ark. (2017) harpagosidin IL-6 ve matriks parçalayıcı proteazlar gibi enflamatuar sitokinlerin üretimini baskılama potansiyelini araştırmak için osteoartritte in vitro bir inflamasyon modeli kullanmışlardır. IL-1β tarafından yüksek oranda indüklenen IL-6 ekspresyonunun, osteoartrit kondrositlerinin harpagosid ile muamele edilmesiyle önemli ölçüde inhibe edildiğini tespit etmişlerdir. Harpagosidin, IL-1β ile indüklenen NF-kappa B (NF-kB) ve C/EBPβ transkripsiyon faktörlerinin aktivasyonunu inhibe etmediğini, ancak Aktivatör Protein-1 (AP-1)'in ana bileşenlerinden biri olan c-FOS'un IL-1β ile tetiklenen indüksiyonunu, fosforilasyonunu ve DNA bağlanma aktivitesini baskıladığını belirlemişlerdir. Ayrıca harpagosidin, patolojik koşullar altında osteoartrit kondrositlerinde MMP-13 ekspresyonunu önemli ölçüde inhibe ettiğini, c-FOS/AP-1 aktivitesini baskılayarak aracılık ettiği enflamatuar uyaranları inhibe ederek önemli bir antienflamatuar etki gösterdiğini öne sürmüşlerdir. Moreau ve ark. (2014) osteoartritli köpeklerde plaseboya kıyasla klinik belirtilerin HP tedavisi ile önemli ölçüde düzeldiğini belirtmişlerdir. Ayrıca, osteoblast hücre kültürlerinde, kemik mineral yoğunluğunun, trabeküler kemik hacminin ve femurdaki trabeküler sayının iyileşmesini önemli ölçüde arttırdığı ve alkalen fosfataz, telopeptid, tartrat dirençli asit fosfataz, osteokalsin, C terminali gibi kemik kaybının biyokimyasal belirteçlerinin serum seviyelerini etkili bir şekilde inhibe ettiği bildirilmiştir (Chung ve ark., 2016).

Inaba ve ark. (2010) yaptıkları çalışmada HP'nin ikincil kökünün %50 etanolik ekstraktının art arda oral uygulanması ile sıçan kronik artrit modelinde önemli bir antienflamatuar etki gösterdiği, HP ekstraktının doza bağlı olarak lipopolisakarit ile indüklenen enflamatuar sitokinlerin [interlökin-1beta (IL-1β), IL-6 ve TNF-α] üretimini baskıladığını tespit etmişlerdir.

Diz veya kalça osteoartritli toplam 122 hastayı içeren bir randomize klinik çalışmada, diaserhein (osteoartrit için yavaş etkili bir ilaç) ve HP ekstraktının oluşan yangıyı önlemede benzer etkinlik gösterdiğini belirtmişlerdir (Chantre ve ark., 2000).

Hu ve ark. (2021) ise spora bağlı hafif/orta derecede boyun/omuz ağrısı olan bireylerde; HP kök ekstresi kremi + standart tedavi (lokal), diklofenak bandı + standart tedavi (lokal) 2 hafta uygulandıktan sonra HP kremi uygulanan grupta ağrı, sertlik, hareketlilik ve çalışma kapasitesinde önemli iyileşme olduğunu gözlemlemişlerdir.

Tapasvi (2022), kuşburnu ekstresi (275 mg), şeytan pençesi ekstresi (100 mg) ve boswellia ekstresinin (50 mg) günde 2 kez 90 gün boyunca lokal olarak kullandığı osteoartritli hastalarda ağrı, hareket kısıtlılığı ve eklem krepiti gibi klinik semptomların tedaviden sonra sırasıyla %62.63, %64.86 ve %69.46 oranında azaldığını tespit etmiştir.

Schopohl ve ark. (2016) harpagidin, hücre göçünü kolaylaştırarak insan monositlerinde ve makrofajlarında güçlü bir bağışıklık modülatörü işlevi gördüğünü ve THP-1 hücre hattında TNF-α salgılamasını azaltabildiği, farklılaşmamış hücrelerde ise lökosit transmigrasyonunda yer alan bazı proteinlerin mRNA ekspresyonunu indüklediğini belirtmişlerdir.

Harpagidin, deri epidermoid karsinomu ve serviks adenokarsinomu hücre hatlarına karşı 90 µg/mL konsantrasyonda dikkate değer antikanser etkiler (%50'nin üzerinde) gösterdiği, fakat aynı konsantrasyonda meme adenokarsinomu hücre hattına karşı daha düşük etkilere (%40'ın üzerinde) sahip olduğu bildirilmiştir (Haznagy-Radnai ve ark., 2008). Mamadalieva ve ark. (2013), yaptıkları çalışmada bu etkileri doğrulamış ve aynı zamanda harpagidin karaciğer kanseri hücre hatlarına karşı da orta düzeyde sitotoksik etkilerinin olduğunu gözlemlemişlerdir.

Kim ve ark. (2003) harpagidin, sıçan kortikal nöron kültürlerinde glutamatla indüklenen oksidatif strese karşı nöroprotektif etki yarattığını tespit etmişlerdir. Harpagidin ayrıca bu hücreleri N-metil-D-aspartat ve kainik asit tarafından indüklenen nörotoksisiteden de koruduğunu ve bu etkinin glutatyonun azalmasını önleyen ve nitrik oksitin aşırı üretimini ve hücresel peroksit seviyesini büyük ölçüde azaltan antioksidatif savunma sisteminin güçlenmesinden kaynaklandığını belirtmişlerdir.

Harpagidin, serebral iskemi-reperfüzyon hasarından sonra sıçan kortikal nöronlarında nöroprotektif etkiler gösterdiği tespit edilmiştir. Aynı zamanda apoptotik hücre ölümünü güçlü bir şekilde önlediği ve bu etkiyi endoplazmik retikulum stresi ile ilgili proteinlerin gen ekspresyon seviyelerinin ve protein üretiminin önemli ölçüde azaltması yoluyla gerçekleştirdiği belirlenmiştir (Wang ve ark., 2020).

Farelerde orta serebral arter tıkanıklığının neden olduğu serebral iskemiden sonra harpagidin hipokampal nöronlar, mitokondriyal fonksiyon ve kaspazdan bağımsız apoptoz yolu üzerindeki etkileri incelenmiştir. Bu durumda apoptozu indükleyen faktörün ekspresyonu ve endonükleaz G ile pro-kaspaz-3 ekspresyonu gözlenerek harpagidin fare beyninin hipokampal nöronlarının apoptoz oranını önemli ölçüde azaltabildiği belirtilmiştir (Zhou ve ark., 2017).

Tang ve ark. (2020) tarafından yapılan çalışmada harpagidin, sinaptik dopamin salınımını büyük ölçüde arttırdığı ve hasarlı nöronlarda normal seviyelere geri döndürdüğü açıkça gösterilmiştir. Sonuç olarak, harpagidin Parkinson hastalığında koruma ve iyileştirme potansiyeline sahip olduğu ancak daha fazla deneysel kanıta ihtiyaç olduğu belirtilmiştir. Yine de harpagidin, hücre içi reaktif oksijen seviyesini etkili bir şekilde hafifleterek α-sinüklein proteininin (Parkinson hastalığının başlamasıyla bağlantılı olan) fosforilasyonunu ve agregasyonunu inhibe ettiği belirtilmiştir.

Sonuç

Günümüzde geleneksel tıpta HP gibi sık kullanılan bitkilere yönelim; düşük yan etki oluşturmaları, klinik ve biyokimyasal faydaları nedeniyle gün geçtikçe artmaktadır. Bitkisel tedavide kullanılan preparatların ilaçlarla olan agonist veya antagonist etkileşimleri, düşük biyoyararlanımları, standardizasyon eksikliği, ülkeler arasındaki farklı düzenlemeler nedeniyle dikkatle kullanılmaları gerekmektedir. Bahsedilen etkilerinden dolayı, şeytan pençesi dahil olmak üzere geleneksel tıpta kullanılan bitkisel ilaçlar hakkında farmakolojik, biyokimyasal ve histolojik değerlendirme de dahil olmak üzere daha fazla araştırma gerekmektedir. Mevcut araştırmalar bitki ekstrelerinin, hastalıkların ilerlemesi üzerindeki etkisini veya belirtileri azaltıp azaltmadığını tam anlamıyla değerlendirememiştir. Bu nedenden dolayı, bitkisel ilaçların etkinliği, güvenliği ve etki mekanizmalarına ilişkin daha kontrollü, moleküler düzeyde kanıtlanmış klinik çalışmalara ihtiyaç vardır.

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Kaynaklar

Açıkgöz Z, Bulut S, Orhan DD, 2020: Harpagophytum procumbens ve fitoterapide kullanımı. *Ankara Ecz Fak Derg,* 44 (3), 540-556.

Anauate MC, Torres LM, De Mello SBV, 2010: Effect of isolated fractions of Harpagophytum procumbens D.C. (devil's claw) on COX-1, COX-2 activity and nitric oxide production on whole-blood assay. *Phytother Res,* 24 (9), 1365–1369.

Anauate MC, Torres LM, de Mello SBV, 2010: Effect of isolated fractions of Harpagophytum procumbens DC (Devil's claw) on COX-1, COX-2 activity and nitric oxide production on whole-blood assay. *Phytother Res,* 24 (9), 1365-1369.

Asadi-Samani M, Bagheri N, Rafieian-Kopaei M, Shirzad H, 2017: Inhibition of Th1 and Th17 cells by medicinal plants and their derivatives: A systematic review. *Phytother Res,* 31 (8), 1128–1139.

Atay I, Kirmizibekmez H, Kaiser M, Akaydin G, Yesilada E, Tasdemir D, 2016: Evaluation of in vitro antiprotozoal activity of Ajuga laxmannii and its secondary metabolites. *Pharm Biol,* 54 (9), 1808–1814.

Betancor-Fernandez A, Perez-Galvez A, Sies H, Stahl W, 2010: Screening pharmaceutical preparations containing extracts of turmeric rhizome, artichoke leaf, devil's claw root and garlic or salmon oil for antioxidant capacity. *J Pharm Pharmacol,* 55 (7), 981–986.

Bindu S, Mazumder S, Bandyopadhyay U, 2020: Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. *Biochem Pharmacol,* 180, 114147.

Chantre P, Cappelaere A, Leblan D, Guedon D, Vandermander J, Fournie B, 2000: Efficacy and tolerance of Harpagophytum procumbens versus diacerhein in treatment of osteoarthritis. *Phytomedicine,* 7, 177–183.

Chicca A, Caprioglio D, Minassi A, Petrucci V, Appendino G, Taglialatela-Scafati O, Gertsch J, 2014: Functionalization of β-Caryophyllene generates novel polypharmacology in the endocannabinoid system. *ACS Chem Biol,* 107, 911499–911507.

Chrubasik JE, Lindhorst E, Neumann E, Gerlach U, Faller-Marquardt M, Torda T, Müller-Ladner U, Chrubasik S, 2006: Potential molecular basis of the chondroprotective effect of Harpagophytum procumbens. *Phytomedicine,* 13 (8), 598-600.

Chung HJ, Kim WK, Park HJ, Cho L, Kim MR, Kim MJ, Shin JS, Lee JH, Ha IH, Lee SK, 2016: Anti-osteoporotic activity of harpagide by regulation of bone formation in osteoblast cell culture and ovariectomy-induced bone loss mouse models. *J Ethnopharmacol,* 179, 66–75.

Cragg GM, Newman DJ, 2013: Natural products: a continuing source of novel drug leads. *Biochim Biophys Acta,* 1830, 3670–3695.

Dragos D, Gilca M, Gaman L, Vlad A, Iosif L, Stoian I, Lupescu O, 2017: Phytomedicine in joint disorders. *Nutrients,* 9 (1), 70.

Georgiev MI, Alipieva K, Orhan IE, 2012: Cholinesterases inhibitory and antioxidant activities of Harpagophytum procumbens from in vitro systems. *Phytother Res,* 26, 313-316.

Gfeller V, Huber M, Förster C, Huang W, Köllner T, Erb M, 2019: Root volatiles in plant-plant interactions I: High root sesquiterpene release is associated with increased germination and growth of plant neighbours. *Plant Cell Environ,* 42, 1950–1963.

Grant L, McBean DE, Fyfe L, Warnock AM, 2007: A review of the biological and potential therapeutic actions of Harpagophytum procumbens. *Phytother Res,* 21 (3), 199-209.

Guan L, Gong D, Yang S, Shen N, Zhang S, Li Y, Wu Q, Yuan B, Sun Y, Dai N, Zhu L, Zou Y, 2018: Genipin ameliorates diet-induced obesity via promoting lipid mobilization and browning of white adipose tissue in rats. *Phytother Res,* 32 (4), 723–732.

Gxaba N, Manganyi MC, 2022: The Fight against Infection and Pain: Devil's Claw (Harpagophytum procumbens) a Rich Source of Anti-Inflammatory Activity: 2011–2022. *Molecules,* 27, 3637.

Haseeb A, Ansari MY, Haqqi TM, 2017: Harpagoside suppresses IL-6 expression in primary human osteoarthritis chondrocytes. *J Orthop Res,* 35 (2), 311–320.

Haznagy-Radnai E, Rethy B, Czigle S, Zupko I, Weber E, Martinek T, Falkay G, Mathe I, 2008: Cytotoxic activities of Stachys species. *Fitoterapia,* 79, 595–597.

Hu S, Belcaro G, Cesarone MR, Feragalli B, Cotellese R, Dugall M, Scipione C, Scipione V, Maione C, Maramaldi G, Togni S, Riva A, 2021: A sport cream (Harpago-Boswellia-ginger-escin) for localized neck/shoulder pain. *Minerva Med,* 112, 255–260.

Huang THW, Tran VH, Duke RK, Tan S, Chrubasik S, Roufogalis BD, Duke CC, 2006: Harpagoside suppresses lipopolysaccharide-induced iNOS and COX-2 expression through inhibition of NF-kappa B activation. *J Ethnopharmacol,* 104 (1-2), 149-155.

Iliev ID, Funari VA, Taylor KD, Nguyen Q, Reyes CN, Strom SP, Brown J, Becker CA, Fleshner PR, Dubinsky M, Rotter JI, Wang HL, McGovern DPB, Brown GD, David M Underhill DM, 2012: Interactions between commensal fungi and the C-type lectin receptor Dectin-1 influence colitis. *Science,* 336, 1314–1317.

Inaba K, Murata K, Naruto S, Matsuda H, 2010: Inhibitory effects of Devil's claw (secondary root of Harpagophytum procumbens) extract and harpagoside on cytokine production in mouse macrophages. *J Nat Med,* 64 (2), 219-222.

Kaszkin M, Beck KF, Koch E, Erdelmeier C, Kusch S, Pfeilschifter J, Loew D, 2004: Downregulation of iNOS expression in rat mesangial cells by special extracts of Harpagophytum procumbens derives from harpagoside-dependent and independent effects. *Phytomedicine,* 11 (7-8), 585-595.

Khumalo GP, Van Wyk BE, Feng Y, Cock IE, 2022: A review of the traditional use of southern African medicinal plants for the treatment of inflammation and inflammatory pain. *J Ethnopharmacol,* 283, 114436.

Kim SR, Koo KA, Sung SH, Ma CJ, Yoon JS, Kim YC, 2003: Iridoids from Scrophularia buergeriana attenuate glutamate-induced neurotoxicity in rat cortical cultures. *J Neurosci Res,* 74, 948–955.

Lim DW, Kim JG, Han D, Kim YT, 2014: Analgesic effect of Harpagophytum procumbens on postoperative and neuropathic pain in rats. *Molecules,* 19 (1), 1060-1068.

Locatelli M, Ferrante C, Carradori S, Secci D, Leporini L, Chiavaroli A, Leone S, Recinella L, Orlando G, Martinotti S, Brunetti L, Vacca M, Menghini L, 2017: Optimization of aqueous extraction and biological activity of Harpagophytum procumbens root on ex vivo rat colon inflammatory model. *Phytother Res,* 31 (6), 937-944.

Mahomed IM, Ojewole JAO, 2005: Analgesic, anti-inflammatory and antidiabetic properties of Harpagophytum procumbens DC (Pedaliaceae) secondary root aqueous extract. *Phytother Res,* 18, 982–989.

Mamadalieva NZ, El-Readi MZ, Ovidi E, Ashour ML, Hamoud R, Sagdullaev SS, Azimova SS, Tiezzi A, Wink M, 2013: Antiproliferative, antimicrobial and antioxidant activities of the chemical constituents of Ajuga turkestanica. *Phytopharmacology,* 4 (1), 1–18.

Mncwangi N, Chen W, Vermaak I, Viljoen AM, Gericke N, 2012: Devil's Claw-a review of the ethnobotany, phytochemistry and biological activity of Harpagophytum procumbens. *J Ethnopharmacol,* 143 (3), 755–771.

Morea, M, Lussier B, Pelletier JP, Martel-Pelletier J, Bedard C, Gauvin D, Troncy E, 2014: A medicinal herb-based natural health product improves the condition of a canine natural osteoarthritis model: A randomized placebo-controlled trial. *Res Vet Sci,* 97, 574–581.

Moura MDG, Lopes LC, Biavatti MW, Kennedy SA, Silva MCO, Silva MT, Bergamaschi CC, 2017: Oral herbal medicines marketed in Brazil for the treatment of osteoarthritis: A systematic review and meta-analysis. *Phytother Res,* 31 (11), 1676–1685.

Nyberg ST, Singh-Manoux A, Pentti J, Madsen IE, Sabia S, Alfredsson L, Bjorner JB, Borritz M, Burr H, Goldberg M, Heikkila K, Jokela M, Knutsson A, Lallukka T, Lindbohm JV, Nielsen ML, Nordin M, Oksanen T, Pejtersen JH, Rahkonen O, Rugulies R, Shipley MJ, Sipila PN, Stenholm S, Suominen S, Vahtera J, Virtanen M, Westerlund H, Zins M, Hamer M, Batty GD, Kivimaki M, 2020: Association of healthy lifestyle with years lived without major chronic diseases. *JAMA Intern Med,* 180, 760–768.

Parenti C, Arico G, Chiechio S, Di Benedetto G, Parenti R, Scoto G, 2015: Involvement of the heme-oxygenase pathway in the antiallodynic and antihyperalgesic activity of Harpagophytum procumbens in rats. *Molecules,* 20 (9), 16758-16769.

Parenti C, Arico G, Pennisi M, Venditti A, Scoto GM, 2016: Harpagophytum procumbens extract potentiates morphine antinociception in neuropathic rats. *Nat Prod Res,* 30 (11), 1248-1255.

Pritsas A, Tomou EM, Tsitsigianni E, Papaemmanouil CD, Diamantis DA, Chatzopoulou P, Tzakos AG, Skaltsa H, 2021: Valorisation of stachysetin from cultivated Stachys iva Griseb. as anti-diabetic agent: a multi-spectroscopic and molecular docking approach. *J Biomol Struct Dyn,* 39 (17), 6452-6466.

Recinella L, Chiavaroli A, Ronci M, Menghini L, Brunetti L, Leone S, Tirillini B, Angelini P, Covino S, Venanzoni R, Zengin G, Simone SD, Ciferri MC, di Giacomo V, Cataldi A, Rapino M, Valerio VD, Orlando G, Ferrante C, 2020: Multidirectional pharma-toxicological study on Harpagophytum procumbens DC. ex Meisn.: An IBD-focused investigation. *Antioxidants,* 9, 168.

Rotunno R, Oppo I, Saetta G, Aveta P, Bruno S, 2018: NSAIDs and heart failure: A dangerous relationship. *Monaldi Arch Chest Dis,* 88, 950.

Sawa T, Nakao M, Akaike T, Ono K, Maeda H, 1999: Alkylperoxyl radical-scavenging activity of various flavonoids and other phenolic compounds: Implications for the anti-tumor-promoter effect of vegetables. *J Agric Food Chem,* 47 (2), 397–402.

Schopohl P, Grüneberg P, Melzig MF, 2016: The influence of harpagoside and harpagide on TNFα-secretion and cell adhesion molecule mRNA-expression in IFNγ/LPS-stimulated THP-1 cells. *Fitoterapia,* 110, 157–165.

Serrano A, Ros G, Nieto G, 2018: Bioactive compounds and extracts from traditional herbs and their potential anti-ınflammatory health effects. *Medicines,* 5 (3), 76.

Stewart KM, Cole D, 2005: The commercial harvest of devil's claw (Harpagophytum spp.) in southern Africa: The devil's in the details. *J Ethnopharmacol,* 100 (3), 225–236.

Tang Y, Yang XK, Zhang XW, Wu WT, Zhang FL, Jiang H, Liu YL, Amatore C, Huang WH, 2020: Harpagide, a natural product, promotes synaptic vesicle release as measured by nanoelectrode amperometry. *Chem Sci,* 11 (3), 778–785.

Tapasvi S, 2022: Clinical efficacy of nutraceuticals in the management of osteoarthritis. *Int J Orthop Sci,* 8 (3), 17-23.

Uchida S, Hirai K, Hatanaka J, Hanato J, Umegaki K, Yamada S, 2008: Antinociceptive effects of St. John's wort, Harpagophytum procumbens extract and grape seed Proanthocyanidins extract in mice. *Biol Pharm Bull,* 31, 240–245.

Ucuncu Y, Celik N, Ozturk C, Turkoglu M, Cetin N, Kockara N, Sener E, Dundar C, Arslan A, Dogan H, Kurt N, Suleyman H, 2015: Chondroprotective effects of a new glucosamine combination in rats: gene expression, biochemical and histopathological evaluation. *Life Sci,* 130, 31-37.

Villasenor IM, 2007: Bioactivities of iridoids. *Anti Inflamm Anti Allergy Agents Med Chem*, 6, 307–314.

Wang K, Lou Y, Xu H, Zhong X, Huang Z, 2020: Harpagide from Scrophularia protects rat cortical neurons from oxygenglucose deprivation and reoxygenation-induced injury by decreasing endoplasmic reticulum stress. *J Ethnopharmacol,* 253, 1–10.

Wegener T, Lüpke NP, 2003: Treatment of patients with arthrosis of hip or knee with an aqueous extract of devil's claw (Harpagophytum procumbens DC.). *Phytother Res,* 17 (10), 1165–1172.

Zhou WB, Lou LY, Zhong MX, Huang Z, 2017: Effects of harpagide on nerve cell and caspase-independent apoptotic signaling pathway in MCAO mice. *Chin Pharmacol Bull,* 33 (12), 1754–1761.

HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Examining Animal Figures in Turkish Mythology in Terms of Veterinary Medicine History

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Abstract: Turks have believed in many religions/beliefs in a long historical process. Among these religions/beliefs, the belief in Shamanism/Sky God has been a priority. This belief, which places nature in its centre, has significantly affected the life and philosophy of life of the Turks. Animals have found their place in the mythology, culture, art, concept of time, literature, and many other subjects of the Turks. The Turks attributed holiness to the horse, the eagle, the deer, and many other animals, especially wolves, and placed them at the centre of their lives. In this study, various important animals for the Turks are discussed in the context of Turkish Mythology and culture; besides the characteristics of the animals, the Turkish Calendar with Twelve Animals and the animals mentioned in the main Turkish Legends (Legend of the Creation, Legend of the Origins, Legend of Oğuz Kağan, Legend of Şu, Legend of Alp Er Tunga, Legend of Satuk Buğra Han) were examined and their importance in terms of the place of animals in the lives of the Turks and the history of veterinary medicine was emphasized.

Keywords: *Animal, History of veterinary medicine, Turkish mythology.*

Türk Mitolojisindeki Hayvan Figürlerinin Veteriner Hekimlik Tarihi Açısından İncelenmesi

Özet: Türkler uzun bir tarihsel süreç içerisinde çok sayıda din ve inanışa inanmışlardır. Bu din ve inanışlar içerisinde Şamanizm/Gök Tanrı inancı öncelikli olmuştur. Doğayı merkezine alan bu inanış Türklerin yaşantısını ve hayat felsefelerini önemli ölçüde etkilemiştir. Hayvanlar, Türklerin mitoloji, kültür, sanat, zaman kavramı, edebiyat ve daha birçok noktasında kendilerine yer bulmuştur. Türkler kurt başta olmak üzere at, kartal, geyik ve daha birçok hayvana kutsallık atfetmiş ve hayatlarının merkezine yerleştirmiştir. Bu çalışmada Türkler için önemli olan çeşitli hayvanlar Türk Mitolojisi ve kültürü bağlamında ele alınmış olup, hayvanların özellikleri yanı sıra On İki Hayvanlı Türk Takvimi ve belli başlı Türk Destanlarında (Yaratılış Destanı, Türeyiş Destanı, Oğuz Kağan Destanı, Şu Destanı, Alp Er Tunga Destanı, Satuk Buğra Han Destanı) adı geçen hayvanlar incelenmiş ve hayvanların Türklerin hayatındaki yeri ve veteriner hekimliği tarihi açısından önemi vurgulanmıştır.

Anahtar Kelimeler: *Hayvan, Türk mitolojisi, Veteriner hekimliği tarihi.*

Introduction

Turks led a nomadic life, mostly based on animals (Koca, 2002), in a geography spanning a wide area, which in history is also designated Eurasia (Baykara, 2001), and with the influence of this wide geography, they believed in many religions/beliefs, primarily Shamanism/Sky God. Nature has shaped the lifestyles, prohibitions, beliefs, and philosophy of Turks who attribute great importance to nature (Güngör, 2002; Moldabay and Azmuhanova, 2019).

Mythology, which is formed by the combination of the Greek words "Mythos" in the sense of fairy tale, story and "Logos" in the sense of science; it is a form of expression that is formed by the emphasization of the supernaturalness of the characters through the creation of events that occurred in primitive ages (Can, 2011; Özarslan, 1994).

In Turkish Mythology, the Sky God, the creator of the universe located in the sky, is referred to as the Turkish God. In addition to the Sky God, there are god/helper spirits with good character known by names such as Ülgen, Kayra Han, Ana Maygil, Yayık, Suyla, Umay, Utkucı, Ak-ene, while there are also god/helper spirits with bad character such as Erlik and Albastı (Çoruhlu, 2002).

Since, throughout history, Turks attribute great importance to nature and animals in their lives, animals have found an important place in Turkish Mythology. While Turks consider some animals sacred, they attribute bad meanings to others (Özkartal, 2012; Tansü and Güvenç, 2015). In Turkish Mythology, animal figures such as the wolf, horse, deer, turtle, ram, sheep, rabbit, eagle, goose, etc., are frequently seen. These animals are frequently referred to in studies on the history of veterinary medicine (Armutak, 2004; Armutak, 2013a; Armutak, 2013b).

Orkun (2022) claims that the first veterinarians in Turks were shamans. In addition to this, he also mentions that in the geographies where Turks live, they have information about animal diseases, especially the horse, and their treatments, which have been passed down from the past to the present. According to Dilgimen (1947), the significance of veterinary medicine among the Turks is emphasized due to their knowledge and usage of words related to animals and diseases. Erk (1966) says that the source of all diseases in ancient Turks was attributed to the spirits and that the shaman also performed rituals in the treatment of animals.

In the study, animal figures frequently encountered in Turkish Mythology: culture, and legends were examined and classified as terrestrial animals, fowls, and other mythological figures. In addition to these, in the study, the Turkish Calendar with Twelve Animals and briefly the animal figures in the main Turkish Legends are also mentioned.

1. Animals

1.1. Terrestrial Animals;

1.1.1. Lion: The Lion figure; although it is thought that it entered Turkish art together with Buddhism, the presence of lion depictions in the Kurgan[1] of Pazyryk[2] shows that the Turks have known the lion since ancient times. The mane of the lion, which is a symbol of bravery, was associated with the Turks because the Turks had long hair (Çoruhlu, 2002). In the west of the Turkish world, the lion is considered a sacred animal. Especially in the Uyghur and Oghuz regions, there are many people named Aslan (meaning lion in the Turkish language). It is possible to say that the lion took the place of the wolf in some Turkish regions. With the advent of Islam, the lion has maintained and continued its importance in society and beliefs with the phrase "Ali, the lion of Allah" (Roux, 2011).

1.1.2. Horse: The Horse**,** as a word, is thought to be one of the oldest words of the Turkish language, it is generally used as a common name among Turks, and it is said to be an animal that Turks have known for a long time. Turks have benefited from horses in many areas, including transportation, meat, and milk, in trade, and especially in wars as passenger animals (Baykara, 2001). While Kaşgarlı Mahmud emphasized the importance of the horse for the Turks with the rhetoric "*The Horse is the Wing of the Turk*", cutting the tail of the horse was designed as "widowing" and was accepted as a sign of mourning. Tying or knotting the tail of a horse is known as war preparation, and this belief continued after Islam. The participation of Sultan Alpaslan in the war by tying the tail of his horse himself before the Battle of Manzikert and his soldiers joining the war by tying the tails of their horses can be given as an example of this. The fact that the tails of horses are found with knots on horse corpses in the kurgans examined in the archaeological excavations shows that this belief has been a tradition that has continued since ancient times (Başdin, 2017; Yılmaz and Toraman, 2020). Although it is certain that the sacrificed horse has a connection with the sky in the Turks, the belief in the steppe in the horse as an animal of celestial origin was common even before Christ (Roux, 2011). It is told that the horse sees the God, and at the same time, in the legends of Yakut, the horse is sent to serve the heroes; in another legend, the devil travels by turning his drum into a three-legged mare, and in the legends of Buryat, he carries the deceased shaman to his new home (Tansü and Güvenç, 2015). The horse is both the animal of death, and the bearer of spirit, and the shaman often used a horse-like pole in his rituals as a tool for the celestial journey. Even in the absence of an available alive horse for the rituals of the shaman, the hair of a grey horse or the skin of a grey mare that he burns is used symbolically for his ritual (Eliade, 1999). "Tulpar", another figure we encounter in Turkish Mythology, is unique to Turkish civilization and mythology, depicted in white or black colours, helping the brave and believed to be faster than the wind. It is a flying horse whose ancestor is believed to be a stallion living in the depths of the waters, and, in the Bashkir

[1] "Kurgan", the typical tomb type of steppe nomads, it is pure Turkish and derives from "korugan". It is thought that this name may have been given because it protects the dead (Tarhan, 2002).

[2] The spread area of Pazyryk Culture is the region that includes the Altai Mountains and its immediate surroundings. Today, it covers the Altai Republic depending on the Russian Federation, the East Kazakhstan Province of Kazakhstan, Western Mongolia and the north of East Turkestan within the borders of China (Kutlu and Kutlu, 2022).

belief, its wings cannot be seen by anyone, and if seen, it will disappear (Gürçay, 2019). Turks, who conferred great importance on horses in their lives and did not spare them, spent most of their time on horseback and discovered and used the saddle before other civilizations. Known for its superior movement and maneuverability, the Turkish Saddle, which has a unique structure, has also found its place in science, and because of its similarity to the Turkish Saddle, its name (*Sella Turcica*) was given to an anatomical structure consisting of the hypophyseal pit (fossa hypophysialis) where the pituitary gland is located on the cerebral surface of the basisphenoid bone and the sellar ridge (dorsum sella) (Gültiken and Osmanağaoğlu, 2006).

1.1.3. Bear: The Bear generally symbolizes the forest spirit in Turkish Mythology. The Bashkirs considered the bear their ancestor, while the Yakuts swore on the bear skull. Shamans kept some of the body parts of the bear on their clothes and used them as a helping spirit in some rituals. Like the wolf, the bear has become taboo in Turkish societies, and saying its name is forbidden (Çoruhlu, 2002). As the mention of the name of the bear is considered objectionable today, the expression "Karaoğlan-Kocaoğlan" (dark boy-big boy) was preferred instead of the bear (Taş, 2020).

1.1.4. Deer: The Deer, is also referred to as "ıwik" in Divan-ı Lugat'it Türk and has been one of the animals that have preserved its importance from past ages to the present day. Deer, which is accepted as a totem animal, also appears as a hunted animal in the Orkhon Monuments. The use of antlers at the entrance of houses in many regions of Anatolia shows that the deer culture from the past has not been forgotten. The deer in Anatolia has been the subject of many legends. For example, it is told that the dervish-ghazi known as "Geyikli Baba" in Bursa took part in the Ottoman Army and participated in battles on his deer in the Ottoman's struggle with Byzantium (Mandaloğlu, 2013). In Turkish Mythology, the deer is mostly passed as good, but it is believed that it will bring bad luck to those who do evil to it. The deer, which helped and guided the shamans and heroes before Islam, also became, without losing its meaning and importance, an animal that guides and protects the saints and heroes with the same importance and meaning after the conversion to Islam of the Turks. It is also said that the deer became immortal by eating the immortal grass on the blessed mountain, known as the land of the sovereigns, and in this way, their existence was continued. Deer is also mentioned as a saving and protective motif in the "Mother Maral Legend" in Cengiz Aytmatov's book "The White Ship" (Aytmatov, 2016).

1.1.5. Tortoise: The Tortoise was described with very similar names in most of the Turkish States [tisbaga (Azerbaijan), tasbaka (Kazakhstan), tasbaka (Uzbekistan), tusbaga, tusbaa, tosbaga (Türkiye)]. Since it has a protective shell, withstands harsh conditions, and is long-lasting, it has also gained an important place in Turkish culture and symbolizes the power, protection, and continuity of the state. The turtle is used in folk medicine against diseases such as infertility, cancer, and tuberculosis, as well as its shell is used against the evil eye and, therefore, hung at the entrance of houses to bring good luck (Ercilasun, 1991; Şimşek, 2016). In addition to these, it is also possible to see a pedestal in the form of a turtle figure in the stones, which have the Göktürk Inscriptions, erected by the Western Turkic Khaganate in the century VIII, and containing a lot of information about the Turks (Derin, 2014).

1.1.6. Ram-Sheep: The Ram-Sheep is an animal that the Turks have attributed various meanings to and sanctified since its apparition in history. Before and after the adoption of Islam, Turks gave importance to the figure of the ram/sheep and placed it at the centre of life by ascribing the meanings of protection, power, strength, bravery, abundance and abundance and protection from the evil eye in the period between birth and death. While stones in the shape of a ram are known as totems in the Turks, in the geographies populated by Turks, the figure of the head of a ram is embroidered like a seal in tombstones to motifs in carpets-rugs (Güven, 2019). In general, although the tradition of erecting stone statues in cult centres and around kurgans, as well as in graves, was especially popular in Turkish societies and widely practised in the centuries VI-XIII, this tradition started to disappear gradually under the influence of Islam (Bağırlı, 2014). On the tombstones with figures such as rams and sheep in Tunceli, and the tombstones with horse figures, historians emphasize that the sacrificial ram and sheep are important for the Turks as well as the sacredness of the horse for the Turks. Accordingly, they think that these figures come from Central Asia and Shamanist culture. Afterwards, they think that this culture continues in Aq Qoyunlu people (Danık, 1993). Ram-sheep-shaped tombstones, as an invariable Turkish culture, have been one of the most important pieces of evidence of the imprints of the Turks in the region, and it is not surprising to encounter horse- and ram-sheep-shaped tombstones in Anatolia. It has been determined by the research that the tombstones found in the geography where the Aq Qoyunlu and Qara Qoyunlu people used to live are quite common in the Turks who lived in Central Asia, Caucasus, and Anatolian lands in the pre-Christian era. There are examples of these tombstones in most of the provinces in Eastern Anatolia, the Eastern Black Sea, and Central and Western Anatolia (Güven, 2019). This connection emerges from the comparison of these tombstones found in Azerbaijan-Nakhchivan and in the province of Tunceli.

1.1.7. Wolf: The Wolf is one of the most important figures in Turkish Mythology. In Turkish Mythology, besides the wolf being a sacred and auspicious animal, it has been described in legends, both male and female, in the role of a parent, a saviour, a leader and guide, a helper of the heroes, brave and compassionate animal. The wolf has also played an important role in folk beliefs and medicine, and it is not considered acceptable to kill wolves or fear them (Uraz, 1994). In most regions of the Turkish world, it is possible to see the god-wolf depiction on the rock tombstone, the materials used by the shaman, and his clothing. The wolf, which is considered to be an element of heaven and earth, was quoted by Ögel (1993) in Turkish cosmology as "*The Small Bear constellation consisted of two horses pulling a chariot and the Big Bear constellation consisted of seven wolves chasing it, and the Yakuts attributed the formation of*

the phases of the moon to the wolves and bears eating the full moon". Although the wolf partially loses its meaning with the acceptance of Islam, its importance continues as a symbol of bravery and power and with the belief that it protects from the evil eye (Çoruhlu, 2002). According to Roux (2011), the wolf plays the most important role in Turkish Mythology. The picture or head of the wolf, which is an ancestor animal, is on the banners, and the marching of the banner-bearer and the wolf leading the army in legends are associated. In connection with this situation, the Göktürks even call the guards of the kagan "wolves" because of their semi-animal nature (Baldick, 2016).

1.1.8. The Cattle, Ox, Yak: The Cattle, Ox, Yak**,** and the combined expressions, it should be first understood that the "Tibetan Ox" is also called yak. This animal, also named "kotas", was hunted as a sacred animal by the Altai Turks, and for this reason, the Old Turks called the drag hunt "cattle". In the Turks, "the drum" and "tug" are signs of khanate, and this sacred tug is made of yak tail. Even in the later periods, when no yak could be found, horses were started to be sacrificed at the feasts (Gökalp, 2014). The bull (ox, cow) is mostly considered a ground element. Due to its power and strength, the bull was accepted as the totem of bravery and the God of war by the Turks. This idea is supported by the comparison of the ruler with a fat, fat bull in the Inscriptions of Tonyukuk (Çoruhlu, 2002).

1.1.9. Rabbit: The Rabbit was counted as an element of earth and sky and it preserved its importance in many Turkish communities, becoming the helping spirit of the shaman, and its skin was used in the drum of the shaman. The rabbit, as it symbolized abundance as a hunting animal among the Göktürks, has been a symbol of abundance and luck in the post-Islamic periods. The tail, head, paw, hide, and ear are the frequently used parts of the rabbit. Rabbit's foot is often used to provide kut[3]. Even though it seems like a necessity to clean the stoves, rabbit feet were used by women in Anatolia mostly for fertility and blessing (Ergun, 2011).

1.1.10. Snake: The Snake has been included in the myths of almost all nations and has mostly been a symbol of medicine (Sivri and Akbaba, 2018). For the Turks, the snake is an animal that has been frequently encountered in both written and oral culture and in pictures since ancient times. In these, very different meanings such as immortality, magic, evil eye, resurrection, underground, holiness, monster, element of earth, and health are ascribed (İnce, 2015). In Shamanism, the snake was associated with the God of the underworld, Erlik, who was said to have a whip made of black blacksnake; and the shaman used the snake as a tool in the rituals (Çoruhlu, 2002).

In Turkish Mythology, the snake is related to medicine and health, and it is mentioned as it follows: "*Akbuğa Khan is the God of medicine and is the protector of physicians. He is symbolized by a large white snake he carries on his arm. The White Snake, on the other hand, unlike the snakes on earth, is not poisonous. Its poison is a medicine and cures all kinds of diseases*" (Karakurt, 2011).

1.2. Fowls;

1.2.1. Goose: The Goose is known as an animal that is thought to have been born in North Asia and is valued by the Turks as a symbol of seigniory and happiness (Sever, 1999). The goose appears as a smart bird in Turkish Mythology. The goose mentors the shaman by showing him which deity and which ways he is able to reach. The shaman uses the goose as a riding animal in some rituals. It is believed that the shaman uses his drum as a horse at the beginning of the ritual, and after a while, by saying that the horse is tired, he turns his drum into a goose and continues his heavenly journey with the goose (İnan, 1986; Sever, 1999).

1.2.2. Crane Bird: The Crane Bird, assumed to be the representative of the Sky God in ancient Turkish belief, is known as the helping spirit of the shaman and is considered sacred. Yakut Turks believe that some of their tribes are descended from this bird. The importance given to the crane has not changed with Islam, and it has preserved its importance as an animal whose coat (don)[4] can be worn. It is rumoured that Ahmed Yesevi also entered the coat of the crane. While murdering a crane is considered bad among the Bashkirs, in Anatolia which is a distant geography, it is believed that it brings disaster to the one who hunts it (Mirzaoğlu, 2019).

1.2.3. Eagle: The Eagle**,** in the context of Turkish Mythology, can be denoted as the grey wolf of the skies. The eagle, which is considered an important symbol of derivation, is the most valuable part of shamanic rituals and one of the national symbols of the Turks. It is thought that the eagle is on the world tree as the representative of the Sky God. It is accepted as a symbol of justice and protection in some Turkish States (Çoruhlu, 2002). The Yakuts attributed the arrival of spring and the melting of snow and ice to the flapping of the wings of an eagle, also called a blackbird. According to the Yakuts, those who take false oaths in the name of the eagle are extinct, and their family ends. According to the belief, childless women beg the eagle and ask him for a child; if the woman had a child, it was believed that the child born was descended from the eagle (İnan, 1986). The eagle, which is important for the Turks, was sometimes used as a double-headed figure. It is thought that the double head represents power and is used in art due to symmetry (Durmuş, 1994). It is possible to encounter the double-headed eagle figure in many architectural monuments in Anatolia.

1.2.4. Falcon: The Falcon is commemorated by Turkish names such as "Tuğrul, Çağrı, Sungur". The Turks took care of this animal mostly in their homes and used it for hunting. Each of the Oghuz tribes used this animal as a symbol. The Turks even think that this animal is an intermediary between the earth and the sky. It is believed that, in a more advanced time, Hacı Bektaş Veli, who came to Anatolia in the coat of a

[3] When people are born they are given a soul and when they die they get it back, but people are not created equal. God also bestowed blessings on his carefully created creatures. This blessing ensured good luck and success in life. (Ögel, 1993).

[4] "A universal motif in which heroes transform into the image of a creature, human, animal or plant so that they can hide or protect themselves" (Ögel, 1993).

pigeon, was fighting with Doğrul Baba, one of the dervishes born in Anatolia, who was in the coat of a falcon. A similar narrative includes parts of Abdulkadir Geylani's life, which Fuad Köprülü mentioned in his work of Kayıkçı Kul Mustafa (Ögel, 1993).

1.3. Other Mythological Figures;

1.3.1. Huma Bird: The Huma Bird is important in terms of Turkish Mythology and often appears in folk music and idioms (state bird, etc.). This bird without feet is always flying and does not land on the ground. It lays eggs in the air and until the egg falls to the ground, it hatches, and a new huma bird is born. According to one narrative, when a society wanted to choose a head for the state, the huma bird was released, and the person whose head was placed on became the head of the state. For this reason, this bird is also known as the state bird. According to a belief, a person who intentionally kills a huma bird dies within forty days (Uraz, 1994).

1.3.2. Phoenix, Simurg, Griffon: The Phoenix, Simurg, Griffon, which appears in different cultures and myths under different names, the Phoenix or Zümrüdüanka bird shows mostly similar characteristics and is generally considered the same as the Sîmurg of Iranian origin and it is also assumed to be related to Garuda in Indian Mythology**.** At the same time, it is thought that this mythological bird may be related to birds of prey and hunter, such as the eagle and the falcon, which have become national symbols for the Turks. The phoenix is a beautifully feathered, long-necked bird. This bird flies very high and takes shelter on Mount Kaf. According to the stories, when death approaches, the sun burns the nest of this bird, and the phoenix re-enters its nest. This bird does not come out of its nest and dies there, and the phoenix is born again from its bones. In pre-Islamic Turkish art, griffons are often depicted as having the body of a winged or wingless lion, the claw and head of an eagle, and the eagle-headed ones, especially unique to the Altai region, represented an important and victorious animal. Gryphons. Although it is a creature that represents the sky, the breaking of dawn, and the sun and points to the element of the Sky (Yang), it also has the characteristics of wisdom, knowledge, enlightenment, alertness, strength, and revenge. Gryphons are also seen as the gathering of the powers of the animals that make up their body. These mingling mythological beings are actually based on the eagle in Turkish Mythology (Çoruhlu, 2019; Uraz, 1994).

1.3.3. Dragon: The Dragon, although widely known in Chinese and Indian circles as an important mythological figure almost all over the world, its importance for the Turks cannot be underestimated. Serpents and lizard-bodied wolves in Siberian and Ordos bronzes may be considered prototypes of the Chinese dragon. The dragon was used in a good sense both in Chinese and Turkish societies. While it was associated with goodness and creative power by the Uyghurs, with Islam, it was thought to represent evil. In the most general sense, it expresses goodness, abundance, prosperity, healing, power, and the cycle of life for the Turks. It is rumoured that, besides abundance, the dragon, as it is associated with the wheel, which has an important place in Turkish art, turning the wheel of the sky. Ejderha found its place in Divanü Lûgat-it-Türk (Çoruhlu, 2019; Saz, 2021).

2. Turkish Calendar with Twelve Animals

Since the old Turks were integrated with nature, this situation also affected their time calculation and calendars. The “Turkish Calendar with Twelve Animals”, also called the “Turkish Calendar”, which started to be used in the religion of Tsin[5], is important for the Turks. The Tsin Turks denominated age to every twelve years in this calendar named with the names of twelve animals. The animals mentioned in the calendar: 1st-year stckan (mouse), 2nd-year ud (cattle, ox), 3rd-year leopard, 4th-year tabışkan (rabbit) 5th-year lu (dragon), 6th-year snake, 7th-year yunt (horse), 8th-year koy (sheep), 9th-year bicin (monkey), 10th-year takagu (chicken), 11th-year it (dog), 12th-year b/ıguz (pig). The person whose year of birth corresponded to an animal was commemorated with the holiness of that animal. The Turks also used this calendar for divination and attributed meaning to every animal. For example, they thought that wars would increase in the year of the cattle due to the fighting of oxen with each other (Gökalp, 2014; Kafesoğlu, 1997; Mahmud, 2005). The Twelve Animal Turkish Calendar was not only used by the Turks throughout history. Apart from the Turks, Mongols, Chinese, Hindus, Tibetan, and Indo-Chinese tribes also used this calendar (Turan, 1941).

3. Legends

According to Yıldız (2009), legends, which is used as a way of expressing mythologies: "They are anonymous works with a unique tradition in which the adventures, world view and ideals of a nation are expressed by uniting them around a hero, typically based on a prototype. Legends are a tool at the most critical point in the formation, protection, and transmission of culture for a nation." When Turkish Legends are examined, various animals are often encountered in their content and this emphasizes the place and importance of animals in Turkish Mythology. To briefly mention these legends:

3.1. The Legend of Creation

The most accepted of the known Creation Legends is the one compiled by Radloff and belonging to the Altai Turks. In the Creation Legend compiled by Radloff, Bay Ulgen is not the great god himself, but a co-creator, and it is possible to see something similar in the Ak-Creator and co-creators of the Yakuts (Ögel, 1993).

In the legend, at the time there was no earth and sky, man and god were flying in the sky. When the time comes, God creates the earth. God says to the bare tree without branches and knots that he sees, "Let nine branches grow from here and become a family" and the devil hears this, he wants them for himself, but God does not give them to him.

[5] According to Gökalp, "Tsinler" is a Turkish tribe that gave its name to China and became the dynasty that united various governments and established an empire for the first time in China (Gökalp, 1976).

When the devil descends to the earth, he says: *"He looked at the people of God, some like humans, some like wild animals, some like birds"*. He thinks of taking them for himself. He sees that man eats fruit from only one branch. The Devil asks man why he does not eat from the others and man says that God forbids it. God puts a dog at the base of this tree as a guard, instructing it not to fall asleep, and a snake next to it, instructing it to sting the devil if he comes. The devil instructs the sleeping snake to eat the fruit and the snake goes and eats the fruit. The devil then tricks two people, Törüngei and Eci. They are normally hairy, but when they eat the fruit they suddenly become hairless, embarrassed and hide behind a tree. God is very angry with these two people and the snake. The snake says, *"O God, I didn't chase you; you had my ear, I was ready for every sound of yours; while I was sleeping here, Devil snuck up on me, he got inside me and made me like this"* and says that Devil deceived him. The serpent says, *" I swear, Devil did not appear to me for some reason, how can I see him?"* God punishes the snake and says, *"O snake, from now on be the Devil himself! Let man be your enemy, let him kill and take your life, be the epitome of evil and let your name remain so!"*. After God punishes the man and woman, it is the devil's turn to be punished. Devil said*: "I asked you to give from your nation, but you ignored my request and told me not to give! "So I stole, deceived, and stole again, and even the one who ran away on a horse, I dropped him and took him again..."*

After the devil has taken up residence in the sky, the devil's sky disintegrates and fragments fall from it, changing the shape of the earth. God throws those who are subject to the Devil out of the sky, "Some drowned in the water, all falling from the sky. Some fell on cattle and died in their horns, some fell on trees and died in their snags..."

The devil asks the heavens for space, but God refuses to give it to him and gives him a space as big as the end of a stick, where the devil begins to spread evil. Realizing the evil intentions of the devil, God gets angry with the devil and says, *"Get down to the bottom of the earth, get down now, get down from here! Take all that you have and all that you don't have, get down from here, get down to the bottom of the earth, and build yourself whatever you want! Gather your roof, your camel, get down to the bottom of the earth!..."*

When the time comes, God gives kut to the devil. *"When he received the kut, the devil withdrew to his world, the devil made a bellows, a pliers. Taking a hammer in his hand, he struck the anvil once, right under the hammer, a frog jumped out. He struck once and a snake came out, wriggled, and disappeared. He struck a bear and it ran away In one shot, the bear came out, ran away, and brownout. Once a pig came out, the wildest of pigs, once an albis came out, the hair of the evil spirit. And out came the şulmus, scattering evil, And out came the camel, and God's patience overflowed. God came angry and gathered everything he had, pliers, hammers, bellows, whatever he had. They were all gathered together and thrown into the fire. From the flame of the bellows, a woman jumped out. From the pliers and the hammer, a man emerged. God was angry and spat in her face. The woman became a bird and flew away, this bird is called Kordoy, it stinks the swamp, and the dog barks at this bird. The male also became a bird, it is called Yalban, its legs are very long and its shape is very strange. This is a very strange bird, for some reason, it has no hands. He searches for his discarded boots. The only thing it loves is gnawing the heel, and the dirt in the house is good food for it".*

God advises his people, *"I have made cattle for you to eat!" "I have made the waters that flow in the world for you, "I have made them pure and clear for you to drink!"* and turning to his helper spirits, he says: *"This is your duty, O Shal-Yime, listen! "Protect those who drink raki and get drunk! "Protect the little children, the lambs! "Protect the little calves, the young foals..."*

And to the god Bodo Sungkü: *"... May orphans find benefit, may they find companionship with everyone! If a Hakan has a good name, don't think, don't believe, that his people are good too! Teach people to be good at all times, teach them how to catch fish with hooks and fish with nets. Teach them how to keep animals, teach them how to keep squirrels, teach them how to make a dress. Always send people on the road better than you! "Train them as if they were me, better than me!"* he advises.

After God had said these things, he said: *"Mani-Shire was all alone in the world. He found a hook and immediately hooked the fishing line, spun the hemp, and made a net himself. He also built a boat and fished with it, the fish were plentiful and he was happy. He invented gunpowder and fired it with a rifle, he used his rifle to hunt squirrels..."* (Ögel, 1993).

3.2. Origin Legend

Although there are various narrations about the Türeyiş saga, three of them are taken from Chinese sources, one narration was examined by Abu'l-Gazi Bahadır Han in his work Şecere-i Türk, and the last and most historical narration was examined by Reşideddin Fazlullah in his work Cami'ut-Tevarih (Bastem, 2019). Ögel (1993) describes the most accepted version of the Derivation Legend in Volume I of his work titled Turkish Mythology as follows:

During the establishment of the Gokturk State, there was no great authority gathering the north of China in its hands. When the Gokturk State was established, all of the surrounding small states began to resemble and get closer to the Turks in order to get the support of the Gokturks.

"The Göktürks, a branch of the Huns, derived from a family called A-si-na (A-shih-na) and later multiplied and began to live in separate tribes. When the Gokturks were defeated by a country called Lin, they were all killed and only a 10-year-old boy survived. The soldiers took pity on the boy and did not kill him, but cut off his feet and threw him among the grasses in the swamp, and left. At this time, a she-wolf appeared around the boy, fed him meat, and raised him, and over time, as the boy grew up, the wolf and the boy began to live together. As a result of this relationship, the she-wolf conceives the child. Hearing that the child was alive, the king of the enemy country immediately ordered his soldiers to find and kill the child. When the soldiers see the boy and the wolf side by side, the wolf immediately escapes from there and goes to the mountain in the north of Kao-ch-'ang

(Turfan), where there is a cave with a plain covered with grass and meadow. Here the pregnant wolf gave birth to ten children. These ten children grow up and breed with the girls they bring from outside. From each of them, a lineage is derived, and the Göktürks are one of these ten lineages" (Ögel, 1993).

3.3. Oğuz Kağan (Oghuz Kaghan) Legend

In the Oğuz Kağan Legend, information about the religion of the Turks is given, the life of the Turks is described and the Töre, the rules of Oghuz, which have shaped the foundation of the Turkish States from the past to the present, are often emphasized.

Much work has been done on the Oğuz Kağan Legend, one of the most important Turkish Legends we have today, and there are two important sources of this legend, one of which is the only manuscript copy in the National Library of Paris, which is incomplete and written in Uyghur script. It was first found by Rıza Nur and published in German by W. Bang and GR. Rahmeti [Arat] in 1932 and then in Turkish in 1936 (Bars, 2008).

The book Oğuz Kağan Legend prepared by Bang and Rahmeti in 1936 begins with the birth of Oğuz Kagan. While explaining that Oğuz Kağan grew up, walked, and started to play forty days after his birth, he also mentions his physical characteristics. He emphasizes the strength and physical structure of Oğuz Kağan by saying that his feet are like ox feet, his waist is like a wolf's waist, his shoulders are like sable shoulders, and his chest is like a bear's chest. Oğuz Kagan herding a herd of horses, rides horses, and hunts prey. At the time when Oğuz Kağan lived, there was a large forest in the region and there were many flying birds in the forest. In this forest, there is a big rhinoceros that eats the herds of horses and the people and torments the people, and Oğuz Kağan goes hunting with the desire to save his people by hunting this rhinoceros. Oğuz Kağan uses deer and bear as bait to catch this wild rhino. Then he stands under the tree and when the rhino comes, he defeats the rhino with his halberd, cuts off its head with his sword, and leaves. When he comes again, he sees a hawk eating the intestines of the rhino and kills the hawk with his bow and arrow.

In the legend, Oğuz Kağan has three sons and Oğuz Kağan gives a big meal to his nation and gathers all the beys at the meal, and makes the following statement: *"I have become Kagan for you; let us take the bow and shield; let us have the aim, buyan; let the wolf (us) uran (war cry)".*

In the legend, while Oğuz Kagan is sleeping in the tent during his expedition to march on Urum Kagan, a light like the sun enters the tent and a wolf with sky feathers and sky mane comes out of that light and addresses Oğuz Kagan: *"You want to march on Urum, I want to march in front of you"* and the wolf marches in front and the soldiers march behind. As a result of the battle, Oğuz Kağan defeats Urum Kağan and makes a treaty with Urum Kağan's brother. Then Oğuz Kağan again sees the wolf with sky feathers and sky mane. The wolf says to Oğuz Kağan: *"You walk from here with the soldiers and take the people and the beys; I will guide you from the front".* Again the wolf walks ahead and the soldiers behind, and Oğuz Kağan is happy.

One day, Oğuz Kağan's beloved pied horse disappeared on the road. The horse escaped to Muz Tagh, a very cold and icy mountain. Among the soldiers, there is a brave soldier who fears neither God nor the devil, and this soldier goes after the horse to this mountain and brings the horse back after nine days. Oğuz Kagan said to this soldier, who was covered with snow, *"You be the head of the beys here and your name will be Karluk forever"* and gave him a lot of jewels and continued to move forward.

One day the wolf stopped again and Oğuz Kağan had his tent pitched in a place called Çürçet, which had horses, oxen, calves, gold, and silver. Çürçet Kagan, who is here, comes against Oğuz Kagan and they start to fight. Oğuz Kağan wins. Oğuz Kağan's soldiers, his retinue, and his people received such a great booty that horses, mules, and oxen were not enough to load and carry it. One of the soldiers, a skillful craftsman, builds a cart and they take all the goods with it.

Oğuz Kağan marched to many places with the wolf was victorious in many battles and annexed many places to his homeland. One day Oğuz Kağan came to a place called Barkan, a warm place with lots of game and birds and lots of gold, silver, and ore. Here he fought with Masar Kağan and as a result of the victory, Oğuz Kağan set off for his homeland with countless goods and horses.

Although the aging Oğuz Kağan wanted to hunt, he called his children because he was old and lacked courage. He instructs three of his children to go east and the other three to go west. After hunting a lot of game and birds, the three of them find a golden bow on the way and give it to their father. Their father cuts the bow into thirds, gives it to his children, and says*: "O my eldest (sons), the bow is yours; shoot arrows like a bow up to the sky"*. After hunting a lot of game and birds, the other children found three silver arrows on the road and gave them to their father. Their father was again delighted, gave the arrows to his children, and said to them: *"O my little (sons), the arrows are yours. The bow shot the arrow: be like the arrow".*

Oğuz Kağan convenes a great assembly and summons his entourage and people. Oğuz Kağan had a golden chicken placed on the pole he had erected on his right side and tied a white sheep under it. He had a silver chicken placed on the pole he erected on his left side and tied a black sheep to the bottom. He sits Buzuks on his right side and Uçoklar on his left side. Oğuz Kağan divided and gave his homeland to his sons and said to his children: *"O my sons, I have travelled a lot, I have seen many battles, I have shot many pikes and many arrows, I have walked a lot on horseback, I have made my enemies cry, I have made my friends laugh. I have paid (my debt) to Sky God. Now I give you my homeland"* and the legend ends by leaving his homeland to his children (Bang and Rahmeti, 1936).

3.4. Şu (Shu) Legend

According to Ercilasun, the source of the majority of Turkish Legends are translations made from the languages of the nations with which they have relations. The Şu Legend is one of them and is found in the "Turkmen" article of Kashgarli Mahmud's Dîvânü Lûgati't-Türk. As mentioned by Kashgarli Mahmud, the time in which the Şu Legend takes place is 300 BC, when Alexander, the famous king of

Macedonia, known as Zülkarneyn, embarked on his expedition to Turkestan (Ercilasun, 2002).

Kashgarli Mahmud narrates the legend as follows:

When Zulkarnayn wanted to pass Samarkand and take the Turkish hands, there was a ruler named Şu. Şu has a silver pool that he carries even during mobilization. He fills this pool with water and releases geese and ducks into it. When the soldiers asked Şu what to do, should we fight, Şu said to his soldiers: *"Look at these geese and ducks, how they dive into the water".* In his words, they got the idea that the sultan was not planning to fight. By this time Zulqarnayn had crossed the river and the scout reported this to Şu. Şu has the drums beaten at night and moves eastward. Thinking that the ruler was unprepared, the people panicked and mounted on whatever beasts they could find, everyone took each other's beasts and they set off. In the morning, the army is formed on a flat plain. When the army suddenly moves out at night, only twenty-two people who cannot find an animal to load their cargo are left. According to the legend, Oghuz tribes are formed from these twenty-two people. Zülkarneyn comes and looks at the people who stayed here, they have long hair and Zülkarneyn immediately realizes that they are Turks. When he saw them, without asking anyone, he called them "Türk mânend", which means "like a Turk". This name remained for those men until today. When the time came and Zulqarnayn retired, 'Şu' returned and went as far as Balasaguna and built a city called Şu. He also put a talisman there. Today, storks come as far as that city, but they cannot pass through it. The effect of this talisman lasts until today (Mahmud, 2005)

3.5. Alp Er Tunga Legend

Although the Iranian poet Firdevsi Persianized Alp Er Tunga in Shahnameh, he mentioned this name a lot (Aksoy, 2002; Atsız, 1992). Alp Er Tunga, who is mentioned as Efrasiyab in Shahnameh, is mentioned by Kashgarli Mahmud (2005) in his work Divânu Lügati't-Türk, who says that Efrasiyab and Alp Er Tunga are the same person. Information about Alp Er Tunga is given in Orkhon Monuments, Kutadgu Bilig, Şeh-nâme, Divanü Lügat-it-Türk, Şecere-i Terâkime and Süleymannâme (Alptekin and Şenocak, 2019). The main source we have is the Shahnameh written by Firdevsi, which talks about the struggle between Turks and Iranians.

In the legend: Upon the death of the Sultan of Iran, the Sultan of Turan, who is also the father of Alp Er Tunga, gathers the elders with the idea of waging war against Iran. Upon the speech of the sultan who says that it is time for the Turk to take revenge, Alp Er Tunga's sense of revenge swells and he says to his father: *"I am the one who can fight with lions, I must take revenge on Iran"*. In addition to Alp Er Tunga's height like a cypress, his chest and arms are like lions, he is as strong as an elephant and his tongue is like a ravenous sword. Alp Er Tunga's brother was hesitant about the war, but his father told him, *"Alp Er Tunga is a lion in hunting and an elephant in war. The knight is a crocodile. He is avenging his ancestors. You should unite with him. When the grass grows green in the plains, march your army to "Amul". Trample Iran with your horses. Paint the waters with blood,"* he protests.

Alp Er Tunga moves to Iran with his army, when the two armies meet, the Turkish warrior Barman asks for an equal soldier from the other side, and only Kubad from the other side volunteers for this battle. His brother tells him *"Barman is a young, lion-hearted horseman. His neck is as tall as the sun. You are old. If the blood reddens your white hair, our braves will be frightened"*, but Kubad does not listen to him, and says *"Man is the prey, death is his hunter"* and starts the battle and is defeated at the end of the battle.

The Iranian leader directly wants to kill Alp Er Tunga, so he gathers his army and moves on. Meanwhile, Alp Er Tunga was sitting in his headquarters in Beykend with tents made of parskin.

At one point, Alp Er Tunga enters the city, which is crowded and over which not even an eagle flies, with plenty of food and pools. He closes himself in the city and when the time comes, Alp Er Tunga is defeated again and again. At the end of the legend, Alp Er Tunga, who never dies, can only be killed by trickery (Atsız, 1992).

3.6. Satuk Buğra Han Legend

Satuk Buğra Han was born at the end of the IXth century and was the first Muslim ruler of the Karahanid State, which was the first Turkish state to convert to Islam (Hunkan, 2009).

According to the legend: Hazrat Muhammad saw all the prophets during Miraj, but since he could not recognize one of them, he asked Cebrail (Gabriel) about that person. Cebrail tells him that this person is not a prophet, but a person who will come down to earth three centuries after the death of Prophet Muhammad and spread the Religion of Islam in Turkestan. Prophet Muhammad is very happy to hear this and prays for this person. Muhammad prays for this person to appear, and after the prayer, forty horsemen suddenly appear in front of him. These horsemen are the spirits of forty friends with Satuk Buğra Han at their head.

When the son of the ruler of Kashgar, whom he named Buğra, was born, disasters occurred and fortune tellers interpreted this as the baby would grow up to be a Muslim and demanded that he be killed, but his mother opposed this and saved her son.

When he was twelve years old, Satuk Buğra Han went hunting with forty friends, saw a rabbit, and started to chase it. Satuk Buğra Han leaves his friends while chasing the rabbit. After chasing the rabbit for a while, this rabbit, disguised as an old man, is Hızır Alayhisselam and Satuk Buğra Han understands this situation and listens carefully to his advice.

When the time comes, Satuk Buğra Han's father dies and his mother marries his uncle as per custom. Satuk Buğra Han invited his uncle to Islam, but when his uncle refused, his uncle entered the earth and Satuk Buğra Han became the ruler. Satuk Buğra Han started to spread Islam in the Turkish Country, while the fire coming out of his mouth burned the non-believers, his sword extended forty steps when he turned to the enemy. When Satuk Buğra Han reached the age of ninety, everyone in the country became Muslim and he fought with China and spread Islam there.

Satuk Buğra Han returned to Kashgar with a divine order and died there. One of Satuk Buğra Han's daughters was

named Alanur. One day Alanur faints in front of her house, frightened by a lion she sees. When she wakes up, she realizes that she will have a child. They named the child Ali. They gave this name because he was a lion of Allah like Hazrat Ali. According to another manuscript, Alanur became pregnant when a light brought by Gabriel flowed into her mouth. She named her son Seyyid Ali Arslan Han because he was the lion of Allah like Hazrat Ali (Sepetçioğlu, 1998).

Conclusion

In this study, animals, which have an important place in the life and meaning of Turks, are examined in the context of Turkish Mythology and the history of veterinary medicine.

As a result of living a life in a long historical interval and a wide geography, Turks have been in contact with many civilizations, cultures, and religious beliefs. These abstract concepts have been partially changed and passed down from generation to generation. As a result of placing great value on nature in general and animals in particular, many concepts such as philosophy of life, belief, literature, art, architecture, medicine, and veterinary medicine have been shaped in this direction.

Turkish Mythology contains many nature and animal figures in connection with the life of Turks. In this context, the gods and helper spirits in Turkish Mythology were found in the form of formations that helped or persecuted the shaman, humans, and animals, and mediated between the shaman and humans and the Sky God.

Animals have a special importance in the mythology of the Turks. Among these animals, the wolf undoubtedly takes the first place. According to myths, legends, and folklore, the wolf has often become taboo and the object of people's fear. On the other hand, the wolf has been the leader and guide in the figure of mother-father in legends. The wolf adorned the flags, pennants, and banners of the Turks. In our recent history, it has maintained its importance and has been included in the emblems of various institutions and organizations (such as the Institute of Turcology, Turkish Hearths and Petrol Ofisi). The horse, which is defended as "the wing of the Turk", appears as a friend and companion of the Turk. Horses, which were the riding animals of the Shaman on his journey to the sky, were buried in Kurgans with their saddles and harnesses in order to be a companion to their owner after death. The horse and ram, which were also sacrificial animals, also adorned tombstones. The deer, which is in a similar position to the horse, did not lose its value after Islam and became a riding animal for dervishes and scholars. The Yak, a symbol of power and strength, was considered a sign of alliance and sovereignty, and the tail of the yak was frequently used on tughons. The snake, which has an important place in folk beliefs as well as mythology, has been the representative of continuity, immortality, and healing and has represented health both in Turkey and in the world. The bear, on the other hand, was a totem animal like the wolf, and as a result of this taboo, it was considered inconvenient to say its name even today, so the expression "Karaoğlan-Kocaoğlan" was preferred instead of a bear. Sometimes the wolf was replaced by a lion, while the turtle took its place on the basis of the inscriptions as a symbol of permanence and longevity.

Flying animals were likewise important and among them, the eagle positioned itself close to the gray wolf. The eagle symbol is still influential today and as a symbol of power, it has been included in many architectural works as well as in the emblems of various institutions and organizations. The goose, who was a companion and mentor to the shaman on his journey to the sky, was also the representative of principality and sovereignty. The crane, on the other hand, is the animal that Hodja Ahmet Yesevi used to wear, and it is not seen frequently in folk art and practices today.

Other mythological figures are generally mixed with world mythology. Examples include the Huma Bird, known as the bird of state, the Phoenix Bird, the Simurg, known as the Blackbird in Turkish, and the Griffin, which has the characteristics of many animals. The Wyvern or Dragon is a figure that occupies a large place in Asian mythology. This figure has also gained an important place in Turks.

Placing animals at the center of life, the Turks also used animals in their divination and calendars. Each animal in the Twelve Animal Turkish Calendar was attributed a meaning. According to the characteristics of those animals, the Turks predicted what would happen that year and what awaited them.

Although there are many Turkish Legends, certain legends were analyzed in this study. It is possible to encounter many animal figures in the legends included in the study. The animal figures described in the legends show parallel characteristics with the characteristics of animals. In these legends, it is possible to see various land animals (dog, snake, horse, cattle, camel, bear, sheep, lamb, calf, foal, ox, squirrel, wolf, sable, rhinoceros, deer, silver chicken, golden chicken, black sheep and white sheep, lion, elephant, crocodile, pars, rabbit, frog, mule) and winged animals (bird (kordoy, yalban), hawk, goose, duck and stork, eagle).

During excavations and archaeological investigations, saddles and harnesses with animal figures, various animal figurines, human corpses with animal figures, the world's first known carpet, the Pazyryk Carpet with animal figure embroidery, and many other remains were found. In the relic known to the world as the Man in the Golden Robe, a corpse covered with gold plates embroidered with numerous animal figures was found. While turtles are found on the bases of the Göktürk Inscriptions, in the geographical areas where Turks lived, especially in Anatolia, these animal figures have found a place in many architectural works. In addition to these, in Yücel and Sanal's (2021) study on Seljuk Period artifacts, it can be said that Turks prioritized animals in their works even after the adoption of Islam.

Turks used products obtained from animals (such as wolf teeth, nails, hair, and bones; bear nails, teeth, eagle claws, and horse hair) both in human medicine and veterinary medicine. In addition to these, they also used the products obtained from animals for fortune telling to obtain information about the disease status (Bayat, 2002).

Not limited to these, Turks, who spent their lives on horses, slept on horses, ate and drank on horses, designed a

unique saddle and won many battles, and conquered many places with this saddle. This saddle has found its place in the scientific world with the name "sella turcica" based on the Turkish saddle.

As a result, animal figures as an indispensable part of the life of Turks; Turkish culture, daily life, beliefs, written, oral, architectural, and artistic works, and archaeological finds have been examined in the context of Turkish Mythology and the history of veterinary medicine, and the importance of nature and animals for Turks has been emphasized.

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References

Aksoy M, 2002: Destanlarda ve Tarihî Kaynaklarda Alp Er Tunga (Efrâsiyâb). Koca S, Güzel HC (Ed), Türkler Ansiklopedisi 3/21, Yeni Türkiye Yayınları, Ankara, Türkiye.

Alptekin AB, Şenocak E, 2019: Alper Tunga Destanı'nın yeni bir kaynağı olarak: Süleymannâme. *TUDED*, 5(2), 127-143. https://doi.org/10.20322/littera.520641

Armutak A, 2004: Eskiçağ uygarlıklarında kurban edilen hayvanlar üzerine bir inceleme. *İstanbul Üniv Vet Fak Derg,* 30(2), 169-180.

Armutak A, 2013a: Doğu ve Batı Mitolojilerinde hayvan motifi I. memeli hayvanlar. *İstanbul Üniv Vet Fak Derg*, 28(2), 411-427.

Armutak A, 2013b: Doğu ve Batı Mitolojilerinde hayvan motifi II. sürüngenler, balıklar, kanatlılar ve mitolojik hayvanlar. *İstanbul Üniv Vet Fak Derg*, 30(2), 143-157.

Atsız HN, 1992: Türk Edebiyatı Tarihi. Baysan Basım ve Yayın Sanayi, İstanbul, Türkiye.

Aytmatov C, 2016: Beyaz Gemi. Ötüken Neşriyat, Özdek R (Çev) İstanbul, Türkiye

Bağırlı R, 2014: Akkoyunlu ve Karakoyunlu Hanedanlıklar Dönemi at ve koç (koyun) figürlü mezar taşlarının çağdaş seramik sanatı açısından incelenmesi. *Akdeniz Sanat*, 7(13), 48-58.

Baldick J, 2016: Hayvan ve Şaman: Orta Asya'nın Antik Dinleri. 2nd ed., Şahin N, (Çev). Hil Yayın, İstanbul, Türkiye.

Bang W, Rahmeti GR, 1936: Oğuz Kağan Destanı. İstanbul Üniversitesi Yayınevi, Istanbul, Türkiye.

Bars ME, 2008: Oğuz Kağan Destanı üzerine yapılan çalışmalar. *Turkish Studies*, 3/4, 224-240.

Bastem N, 2019: Türeyiş Destanıyla ilgili tüm rivayetlerin karşılaştırılması ve kültürel kodlar üzerine bir inceleme. *Turcology Research*, 0(66), 225-236.

Başdin A, 2017: Selçuklularda İslamiyet öncesi Türk Kültürünün etkileri -Sultan Alp Arslan Dönemi cenaze merasimleri ve gelenekleri-. *Eskiyeni*, (34), 175-190.

Bayat AL, 2002: İslâm Öncesi Orta Asya Türk Dünyasında tababet. Koca S, Güzel HC (Ed), Türkler Ansiklopedisi 3/21, Yeni Türkiye Yayınları, Ankara, Türkiye. s. 449-459

Baykara T, 2001: Türk Kültür Tarihine Bakışlar. Atatürk Kültür Merkezi Yayınları, Ankara, Türkiye.

Can Ş, 2011: Klasik Yunan Mitolojisi. 19th ed., Ötüken Neşriyat, İstanbul, Türkiye.

Çoruhlu Y, 2002: Türk Mitolojisinin Anahatları. Kabalcı Yayınevi, Istanbul, Türkiye.

Çoruhlu Y, 2019: Türk Sanatında Hayvan Sembolizmi. 3rd ed, Ötüken Neşriyat, Istanbul, Türkiye.

Danık E, 1993: Koç ve At Şeklindeki Tunceli Mezartaşları. 2nd ed. Türk Kültürünü Araştırma Enstitüsü Yayınları, Ankara, Türkiye.

Derin S, 2014: Göktürk Kitabelerinde Türk Dini İnancının izleri. *Turkish Studies*, 9(9), 465-474.

Dilgimen H, 1947: Veteriner Hekimliği Tarihi. Bozkurt Matbaası, İstanbul, Türkiye.

Durmuş İ, 1994: Türklerin kullandığı armalar. *Milli Folklor,* 6(21), 40-44.

Eliade M, 1999: Şamanizm. (İ. Birkan, çev.), İmge Kitabevi Yayınları, Ankara, Türkiye.

Ercilasun AB, 1991: Karşılaştırmalı Türk Lehçeleri Sözlüğü. Kültür Bakanlığı Yayınları, Ankara, Türkiye.

Ercilasun AB, 2002: İslâmiyet Öncesi Türk Destanları (Sakaoğlu and Duymaz, Eds.). Ötüken Neşriyat.

Ergun P, 2011: Alevilik-Bektaşilikteki tavşan inancının mitolojik kökleri üzerine. *Turk Kult. ve Haci Bektas Veli - Arast. Derg.,* 19(60), 281 – 312.

Erk N, 1966: Veteriner Tarihi. Ankara Üniversitesi Basımevi, Ankara, Türkiye.

Gökalp Z, 1976: Türk Medeniyet Tarihi. Güneş Matbaacılık, Istanbul, Türkiye.

Gökalp Z, 2014: Türk Töresi. Ötüken Neşriyat, Istanbul, Türkiye.

Gültiken E, Osmanoğlu Ş, 2006: Origin of Sella Turcica. XXXVII International Congress of the World Association for the History of Veterinary Medicine, Spain.

Güngör H, 2002: Eski Türklerde Düşünce, Din ve Bilim. Koca S, Güzel HC (Ed), Türkler Ansiklopedisi 3/21, Yeni Türkiye Yayınları, Ankara, Türkiye.

Gürçay S, 2019: Mitolojilerde uçan at motifi üzerine karşılaştırmalı bir çalışma. *Aydın Türklük Bilgisi Dergisi*, 5(8), 37-54.

Güven O, 2019: Ahıska'dan Artvin'e koç-koyun formlu mezar taşları. *GTTAD*, 4(1), 345-363.

Hunkan ÖS, (2009). Satuk Buğra Han. In TDV İslâm Ansiklopedisi (Vol. 36): Diyanet Vakfı Yayınları, İstanbul

İnan A, 1986: Tarihte ve Bugün Şamanizm Materyaller ve Araştırmalar. 3.ed, Türk Tarih Kurumu Basımevi, Ankara, Türkiye.

İnce C, 2015: Türk Mitolojisinde yılan ve resim sanatındaki izdüşümleri. Yayımlanmamış Yükseklisans Tezi, SÜ Sosyal Bilimler Enstitüsü, Konya.

Kafesoğlu İ, 1997: Türk Milli Kültürü. 16. ed. Ötüken Neşriyat, Istanbul, Türkiye.

Karakurt D, 2011: Türk Söylence Sözlüğü. (e-book), Türkiye. Access address; https://upload.wikimedia.org/wikipedia/commons/0/00/TurkSoylenceSozlugu.pdf Date of access; 09.01.2023.

Koca S, 2002: Eski Türklerde Sosyal ve Ekonomik Hayat. Koca S, Güzel HC (Ed), Türkler Ansiklopedisi 3/21, Yeni Türkiye Yayınları, Ankara, Türkiye.

Kutlu M, Kutlu L, 2022: Pazırık Kültüründe ölü gömme gelenekleri ve kurgan mimarisine yansımaları. *Sanat Tarihi Dergisi,* 31(1), 321-353.

Mahmud K, 2005: Divanü Lugati't Türk. Erdi S, Yurteser ST (Çev). Kabalcı Yayınevi, Istanbul, Türkiye.

Mandaloğlu M, 2013: Türk Mitolojisinden Anadolu'ya taşınan kültür: geyik motifi. *J. Int. Soc. Res.,* 6(27), 382-391.

Mirzaoğlu G, 2019: Anadolu Turnaları Biyoloji, Kültür, Koruma. Özdağ U, Alpaslan GG (Ed), Ürün Yayınları, Ankara, Türkiye.

Moldabay T, Azmuhanova A, 2019: Eski Türklerin Dini. *Motif Akademi Halkbilimi Dergisi*, 12(27), 894-901.

Orkun HN, 2022: Eski Türklerde Evcil Hayvanların Tarihçesi. 1. ed., Demir, N. Altınordu Yayınları, Ankara, Türkiye.

Ögel B, 1993: Türk Mitolojisi. 2nd ed., Türk Tarih Kurumu, Ankara, Türkiye.

Özarslan M, 1994: Mitoloji üzerine araştırmalar. *Milli Folklor*, 3(24).

Özkartal M, 2012: Türk Destanlarında geçen halı anlatımları, halılardaki hayvan motifleri ve renklerinin dili. *Arış Dergisi*, (8), 92-101.

Roux JP, 2011: Eski Türk Mitolojisi. (MY Sağlam, çev.) BilgeSu Yayıncılık, Ankara, Türkiye.

Saz S, 2021: Anadolu'da Türk Sanat ve Mimarisinde rehberlerin karşılaştıkları hayvan figürleri ve mitolojik arka planı. *Uluslar Türk Dünya Turizm Araşt Derg.*, 6(1), 29-46.

Sepetçioğlu MN, 1998: Karşılaştırmalı Türk Destanları 6 ed., İrfan Yayımcılık, İstanbul, Türkiye.

Sever M, 1999: Türk Mitolojisinde kuşlar. *Milli Folklor*, 42, 82-83.

Sivri M, Akbaba C, 2018: Dünya mitlerinde yılan. *Folklor/Edebiyat*, 24(96), 53-64.

Şimşek E, 2016: Türk Kültüründe, kaplumbağalarla ilgili efsaneler üzerine bir değerlendirme. *AKRA JOURNAL*, 3(11), 51 – 63.

Tansü YE, Güvenç B, 2015: Eski Türk Mitolojisinde hayvan motifleri üzerine düşünceler. *Türk Dünyası Araştırmaları*, 111(218), 203-218.

Tarhan MT (2002). Ön Asya dünyasında ilk Türkler: Kimmerler ve İskitler. Koca S, Güzel HC (Ed), Türkler Ansiklopedisi 1/21, Yeni Türkiye Yayınları, Ankara, Türkiye.

Taş E, 2020: Türk halk kültüründe "baş". *Motif Akademi Halk Bilimi Dergisi*, 13(29), 87-108.

Turan O, 1941: Oniki Hayvanlı Türk Takvimi. Ötüken Neşriyat, İstanbul, Türkiye.

Uraz M, 1994: Türk Mitolojisi. 2nd ed., Düşünen Adam Yayınları, İstanbul, Türkiye.

Yıldız N, 2009: Türk destancılık geleneği. *Journal of Modern Turkish Studies*, 6(1), 7-15.

Yılmaz A, Toraman A, 2020: Some examinations on the tradition of horsetail tying and cutting in Turkish Culture. *History Studies*, 12(3), 745-763.

Yücel M, Sanal Ş, 2021: Selçuklu Dönemi eserlerinde hayvan sembolleri: Ahlat Müzesi örneği. *Mersin University School of Medicine Lokman Hekim Journal of History of Medicine and Folk Medicine*, 11(3), 535-545.

HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ YAYIN KURALLARI *

1- Harran Üniversitesi Veteriner Fakültesi Dergisi (Harran Üniv Vet Fak Derg), özellikle Veteriner Hekimliği bilim alanı ile ilgili olmak üzere insan ve hayvan sağlığını kapsayan Türkçe ve İngilizce olarak hazırlanmış orijinal klinik ve deneysel araştırmalar, olgu sunumları, derlemeler (çağrılı veya sorumlu yazara ait derleme konusu ile ilgili en az 3 araştırma makalesinin referans listesinde olması gereklidir), kısa bilimsel makale ve editöre mektuplar yayınlayan hakemli bir dergidir. Dergide İngilizce hazırlanmış makalelerin yayımlanmasına öncelik verilir. Dergi 6 ayda bir, yılda 2 sayı olarak yayınlanır. Yayınlanan makalelerden ücret alınmamaktadır.

2- Dergiye kabul edilen yayınlar başka bir yerde yayınlanmamış olmalıdır. Eş zamanlı olarak incelenmek üzere başka dergilere gönderilmiş olmamalıdır. Yayınlanan makalelerden doğacak her türlü hukuki ve cezai sorumluluk yazarlara aittir. Yazarlara yayın hakkı bedeli ödenmez. Gönderilen makaleler ve ekleri makale yayınlansın veya yayınlanmasın geri iade edilmez.

3- Daha önce kongrelerde tebliğ edilmiş ve özeti yayımlanmış çalışmalar, bu durum kapak sayfasında belirtilmek üzere kabul edilir. Bununla birlikte yayın, tezden üretilmiş ise ve destekleyen kuruluş var ise yayında belirtilmelidir.

4- Dergi Editörlüğüne ulaşan makale, dergi editörlüğünce ön değerlendirmeye tabi tutulur. Editörlük, ön değerlendirme sonucuna göre makaleyi reddetme veya hakem değerlendirmesine tabi tutmadan önce düzeltme isteme hakkına sahiptir.

5- Makaleler değerlendirme için en az iki hakeme gönderilir. Makale kabul sürecinde, iki hakemin görüşlerinin farklı olması durumunda editör, üçüncü bir hakemin veya danışma kurulunun görüşünü alarak karar verir.

6- Harran Üniversitesi Veteriner Fakültesi Dergisi, etik ilkelere saygı çerçevesinde, TÜBİTAK ULAKBİM tarafından Türkiye'de tüm üniversitelerin kullanımına açmış olduğu "ithenticate" intihal tespit programı aracılığıyla gönderilen tüm makale, olgu sunumu ve derlemelerin ön değerlendirmesinin yapılması ve sonuçların gönderilmesi gerekmektedir. Bu ön değerlendirme sonuçlarına göre, makale, olgu sunumu veya derlemelerin başka kaynaklarla benzerlik oranının **%15'i** (özet, abstract ve kaynaklar hariç) aşmaması gerekmektedir. "ithenticate" programı aracılığı ile yapılacak öndeğerlendirmede benzerlik oranının %15 değerini aşması durumunda yayımlanmak üzere dergimize gönderilen makale, olgu sunumu veya derlemeler değerlendirilmeye alınmayacaktır.

7- Gönderilen herhangi bir makalenin (tüm makale kategorileri için) referanslarının en az % 20'sinin son beş yılda yayınlanan referansları içermesi gerekir. Anonim kaynaklar asgari düzeyde tutulmalıdır.

8- Makale yayına kabul edildiği takdirde her türlü yayın hakkının devredildiğine dair beyanları kapsayan Telif Hakkı Devir Sözleşmesinin tüm yazarlar tarafından imzalanarak basımdan önce elektronik olarak dergi editörlüğüne gönderilmesi gerekmektedir. Telif Hakkı Devir Sözleşmesi gönderilmeyen makaleler yayımlamaya kabul edilmiş olsalar bile basılmazlar.

9- Harran Üniversitesi Veteriner Fakültesi Dergisi'ne gönderilecek makale, olgu sunumu, derleme vb. çalışmalar, https://dergipark.org.tr/tr/pub/huvfd adresinden gönderildiğinde değerlendirme sürecine alınmaktadır.

10- Harran Üniversitesi Veteriner Fakültesi Dergisi'ne gönderilecek makale, olgu sunumu, derleme vb. çalışmalar MS Word formatında, tüm fotoğraflar (resimler) en az 300 dpi çözünürlükte, TIFF veya JPEG formatında kaydedilmiş olmalıdır.

YAZIM KURALLARI

Yazılar, MS Word formatında, Times New Roman yazı tipinde, 12 punto, çift satır aralıklı ve her kenardan 2.5 cm boşluk bırakılarak hazırlanmalıdır. Makaleye satır numaraları (makalenin 2. sayfasından başlamak üzere sürekli olacak şekilde) eklenmelidir. Bu şekildeki yazılar, şekil ve tablolar dâhil olmak üzere orijinal bilimsel araştırmalar ve derlemelerde 15, kısa bilimsel makale ve olgu sunumlarında 5 sayfayı geçmemelidir.

Birimler ve ölçüler için Uluslararası Standart birimleri (SI-sistem) kullanılmalıdır.

Araştırma Makaleleri: Orijinal araştırma makaleleri aşağıdaki ana konu sıralamasına göre dizilmelidir: Başlık, Yazar adları (Sorumlu yazar (*) ile işaretlenmeli), Yazar adresleri, Yazar ORCID numaraları, Özet ve Anahtar kelimeler (3 - 6 kelime), İngilizce başlık, Abstract ve Keywords ile Giriş, Materyal ve Metot, Bulgular, Tartışma ve Sonuç, Teşekkür veya Bilgilendirme ile Kaynaklar. Her bir Tablo ve Şekil ayrı sayfalarda yer almalıdır.

YAZIM DÜZENİ

Özet: Orijinal araştırma makalelerinde 250, diğer makale türlerinde 200 kelimeyi geçmeyecek şekilde hazırlanmalıdır.

Anahtar Kelimeler: En fazla 6 tane olmak üzere her iki dildeki özetin altında alfabetik sırayla verilmelidir. Anahtar kelimeler, Türkiye Bilim Terimleri arasından seçilmelidir. Anahtar kelimelerin seçiminde Türkiye Bilim Terimleri internet adresinden (http://www.bilimterimleri.com) yararlanılmalıdır.

Giriş: Sonuçların anlaşılabilmesi ve yorumlanabilmesi için o konu ile ilgili yapılmış olan çalışmalar hakkında bilgilere yer verilmelidir. Giriş'te çalışmanın hipotezi belirtilmelidir. Çalışmanın amacı bu bölümün en sonunda açık olarak yazılmalıdır.

Materyal ve Metot: Bu bölümde deneysel çalışmalar diğer araştırmacılar tarafından tekrarlanabilecek yeterlilikteki detayı ile verilmelidir. Uluslararası indeksli dergilerde yayınlanmış bir makalede açıklanan bir teknik kullanıldığında, metodun çok kısa açıklanması ve ilgili orijinal makaleye atıf yapılması gereklidir. Makalede etik kurul izini ve/veya yasal/özel izin alınmasının gerekip gerekmediği bu bölümde belirtilmelidir. Materyal olarak hayvan kullanılan orijinal araştırma makalelerinde (klinik, deneysel, saha çalışmaları vb.); etik kurul onayı alınmış olmalıdır. Etik kurul onay/izin belgesinin "alındığı etik kurulun ismini, sayısını ve tarihini" içeren açıklayıcı bilgiler materyal ve metot bölümüne yazılmalıdır. Yayın kurulu etik kurul onay belgesini isteme hakkına sahiptir.

Bulgular: Araştırma bulguları açık ve anlaşılabilir şekilde verilmelidir. Bulgular, gerektiğinde tablo ve şekillerle desteklenmeli ve kısa olarak sunulmalıdır.

Tartışma ve Sonuç: Bulgular gereksiz ayrıntıya girmeden literatürler ışığında tartışılmalı ve bulguların önemi vurgulanmalıdır. Sonuç ya da öneri cümlesi ile bitirilmelidir.

Teşekkür: Çalışma veya makaleye kişisel katkı ve parasal destek burada belirtilmelidir.

Derleme: Derginin yayın alanlarındaki konularda yenilikleri içeren, güncel kaynaklardan yararlanılarak hazırlanmış makaleler olup, yazarların konu ile doğrudan ilişkili en az 3 adet çalışmalarının olması ve bunların derleme içinde kullanılması durumunda yayınlanmak üzere kabul edilebilecektir. Sorumlu yazar, derlemesini gönderirken konu ile ilgili makalelerinin de künye bilgilerini dergi editörlüğüne göndermelidir (makale künyeleri, makale metninin en son sayfasında sunulmalıdır). Harran Üniversitesi Veteriner Fakültesi Dergisi'nde değerlendirmeye alınan ve yayınlanan derlemeler **çağrılı derleme**lerden oluşmaktadır. Derlemelerde; Özet, Giriş, Sonuç ve Kaynaklar bölümleri bulunmalıdır.

Olgu Sunumu: Yazarların, karşılaştıkları yeni veya ender gözlemlenen olguların ele alındığı, bilimsel değere sahip bilgileri içeren eserlerdir. En fazla 15 kaynak kullanılmalı ve bu kaynakların güncel olmasına özen gösterilmelidir. Olgu sunumları; Özet, Giriş, Olgu tanımı, Tartışma ve Sonuç ile Kaynaklar bölümlerinden oluşmalıdır.

Kısa Bilimsel Makale: Kısa bilimsel makalelerde dar kapsamlı olarak ele alınmış, yeni bilgi ve bulgular sunulmalıdır. Araştırma makalesi formatında hazırlanmalı ve en fazla 5 sayfa olmalıdır. En fazla 2 tablo veya şekil içermelidir.

Kaynaklar

Metin içinde atıf yapılırken;

1. Yazar veya yazarların soyadından sonra parantez içinde kaynağın yayın yılı belirtilmelidir; Adams (1998) tarafından; Wilkie ve Whittaker (1997) tarafından; Doyle ve ark. (2007) tarafından….
2. Cümlenin sonunda atıf yapıldığında ise yazar ismi ve yayın yılı parantez içinde belirtilmelidir; ... bildirilmiştir (Adams, 1998); .... bildirilmiştir (Wilkie ve Whittaker, 1997); ..... bildirilmiştir (Doyle ve ark., 2007).
3. Birden çok kaynağa atıf yapılması durumunda önce alfabetik sonra kronolojik sıralama yapılmalıdır;
   …. bildirilmiştir (Adams, 1998; Adams, 2008; Doyle ve ark., 2007; Wilkie ve Whittaker, 2006).
4. Aynı yazarın aynı yıl yayınları söz konusu ise her biri "a" harfinden başlayarak küçük harflerle işaretlenmelidir;
   …. (Adams, 2005a; Adams, 2005b;…).

Kaynak listesi aşağıdaki şekilde hazırlanmalıdır:

1. **Kaynak listesi yazar soyadına göre alfabetik olarak sıralanmalıdır.**
2. **Kaynaklarda yer alacak dergi adları ISI web of Science'a göre kısaltılmalı ve italik yazılmalıdır.**
3. **Kaynakların yazın şekli aşağıdaki şekilde olmalıdır.**

Makale; Sullivan JC, Sasser JM, Pollock JS, 2007: Sexual dimorphism in oxidant status in spontaneously hypertensive rats. *Am J Phsiol Integr Comp Physiol,* 292 (1), 64-68.

Kitap; Cadenas E, Packer L, 2001: Handbook of Antioxidants. 2nd ed., Marcel Dekker Inc., New York, USA.

Kitaptan bir bölüm: Bahk J, Marth EH (1990). Listeriosis and Listeria monocytogenes In: Foodborne Diseases, Cliver DO (Ed), 248-256, Academic Press, San Diego. **Web sayfası:** Anonim (1) http://www.emea.europa.eu, Erişim tarihi; 01.04.2010.

Tez: Er A, 2009: Makrolid grubu antibiyotiklerin endotoksemide sitokin düzeylerine etkisi. Doktora tezi, SÜ Sağlık Bilimleri Enstitüsü, Konya.

Bilimsel toplantıda sunulan bildiri: Allen WR, Wilsher S, Morris L, Crowhurst JS, Hillyer MH, Neal HN, 2006: Re-establishment of oviducal patency and fertility in infertile mares. In: Proceedings of the Ninth International Symposium on Equine Reproduction, Kerkrade, Holland, pp. 27-28.

Tablo ve Şekiller: Her bir tablo ve şekil ayrı sayfalara yerleştirilmelidir. Kullanım sırasına göre numaralandırılmalı, kısa başlıklarla ifade edilmeli ve metin içinde tablo numarası verilerek atıfta bulunulmalıdır. Tablo başlıkları makalenin yazım dilinde tablonun üst bölümüne yazılmalıdır. Tabloda kullanılan kısaltmalar ve gerekli açıklamalar tablo altında verilmelidir. Şekil başlıkları makalenin yazım dilinde şeklin alt bölümüne yazılmalıdır.

HARRAN UNIVERSITY VETERINARY FACULTY JOURNAL PUBLICATION RULES *

1- Journal of Harran University Veterinary Faculty is a refereed journal that publishes original clinical and experimental research in Turkish and English, covering human and animal health, especially in the field of Veterinary Science, case reports, reviews (at least 3 on the subject of compilation of the invited or responsible author) the research paper must be on the reference list), short scientific articles and letters to the editor. Publishing articles in English is given priority in the journal. The journal is published electronically every 6 months and 2 issues a year. There are no fees for published articles.

2- Publications accepted to the journal should not have been published elsewhere. It should not have been submitted to other journals for review simultaneously. All legal and criminal liability arising from the published articles belong to the authors. Authors are not paid the right to publish. Submitted articles and their attachments are not returned, whether the article is published or not.

3- The works that have been previously communicated in congresses and published with a summary are accepted to be stated on the cover page. However, if the publication is produced from the thesis and there is a supporting organization, it should be stated in the publication.

4- The article that reaches the Journal Editor is subjected to preliminary evaluation by the journal editor. Editing has the right to reject the article according to the preliminary evaluation result or to request correction before subjection to the reviewer.

5- Articles are sent to at least two referees for evaluation. In the article acceptance process, if the opinions of the two referees differ, the editor decides by taking the opinion of a third referee or advisory board.

6- TUBITAK ULAKBIM respect for ethical principles, which opened to the use of all universities in Turkey "iThenticate" sent through plagiarism detection program, the whole article, must be made of the case report and review of the preliminary assessment and the delivery of results. According to these preliminary evaluation results, the similarity rate of articles, case reports or reviews with other references should not exceed 15% (excluding abstract, abstract and references). Articles, case reports or reviews sent to our journal for publication will not be evaluated if the similarity rate exceeds 15% in the evaluation made through the "ithenticate" program.

7- At least 20% of references to any submitted article (for all article categories) must include references published in the last five years. Anonymous references should be kept to a minimum.

8- If the article is accepted for publication, the Copyright Transfer Agreement, which includes the declaration that any right of publication has been transferred, must be signed by all authors and sent to the journal editor before printing. Articles without Copyright Transfer Agreement are not published even if they are accepted to publish.

9- An article, case report, review etc. to be sent to Harran University Veterinary Faculty Journal. When the works are sent to https://dergipark.org.tr/tr/pub/huvfd, they are taken into the evaluation process.

10- An article, case report, review etc. to be sent to Harran University Veterinary Faculty Journal. Works must be saved in MS Word format, all photographs (pictures) at least 300 dpi resolution, in TIFF or JPEG format.

WRITING RULES

Manuscripts should be prepared in MS Word format, Times New Roman font, with 12 font size, double line spacing and 2.5 cm space on each side. Line numbers (continually starting from page 2 of the article) should be added to the article. Articles of this type should not exceed 15 pages in original scientific research and reviews, including figures and tables, and 5 pages in short scientific articles and case reports.

International Standard Units (SI-system) should be used for units and dimensions.

Research Articles: Original research articles should be arranged in the order of the following main topics: Title, Author names (must be marked with the responsible author (*)), Author addresses, Author ORCID numbers, Abstract and Keywords (3 - 6 words), English title, Abstract and Introduction to Keywords, Material and Method, Results, Discussion and Conclusion, Thanks or Information and References. Each Table and Figure should be on separate pages.

STYLE AND FORMAT

Abstract: It should be prepared not to exceed 250 words in original research articles and 200 words in other types of articles.

Keywords: It should be given in alphabetical order below the summary in both languages, maximum 6. Keywords should be selected from Turkey Science Terms. Turkey Science Terms in the selection of keywords from the internet address (http://www.bilimterimleri.com) should be utilized.

Introduction: In order for the results to be understood and interpreted, information about the studies done on that subject should be included. In the introduction, the hypothesis of the study should be specified. The purpose of the study should be clearly written at the end of this section.

Material and Method: Experimental studies should be given in this section with sufficient detail that can be repeated by other researchers. When using a technique described in an article published in international indexed journals, it is necessary to describe the method very briefly and to cite the relevant original article. In the article, it should be stated in this section whether the ethical committee permission and / or legal / special permission should be obtained. In original research articles using animals as materials (clinical, experimental, field studies, etc.); ethics committee approval must have been obtained. Explanatory information including the name, number and date of the ethics committee's ethics committee approval / permit document should be written in the material and method section. The editorial board has the right to request the ethics committee approval document.

Results: Research findings should be given clearly and understandably. Findings should be supported with tables and figures when necessary and presented briefly.

Discussion and Conclusion: Findings should be discussed in the light of the literature before going into unnecessary detail and the importance of the findings should be emphasized. It should be finished with a conclusion or suggestion sentence.

Acknowledgment: Personal contribution and monetary support to the study or article should be stated here.

Compilation: These are articles that contain innovations on the subjects of the journal's publications and are prepared by using current references. If the authors have at least 3 works directly related to the subject and they can be accepted for publication. When submitting his review, the responsible author should send the imprint information of the articles related to the subject to the editor of the journal (article tags must be presented on the last page of the article text). Reviews compiled and published in Harran University Veterinary Faculty Journal are invited reviews. in the compilation; Summary, Introduction, Conclusion and References sections should be available.

Case Report: These are the works that contain information of scientific value that the authors discuss the new or rare cases that they encounter. Maximum 15 references should be used and care should be taken to keep these references up to date. Case reports; It should consist of Summary, Introduction, Case description, Discussion and Conclusion and References sections.

Short Scientific Article: In short scientific articles, it should be handled narrowly and new information and findings should be presented. It should be prepared in the form of a research paper and should not exceed 5 pages. Must contain no more than 2 tables or figures.

References:

While citing in the text;

1. The publication year of the reference should be specified in parentheses after the surname of the author or authors; By Adams (1998); By Wilkie and Whittaker (1997); Doyle et al. (2007) by….

2. When cited at the end of the sentence, the name of the author and the year of publication must be indicated in parentheses; ... have been reported (Adams, 1998); .... has been reported (Wilkie and Whittaker, 1997); ..... has been reported (Doyle et al., 2007).

3. In case of reference to more than one reference, first alphabetical and chronological order should be done;

.... reported (Adams, 1998; Adams, 2008; Doyle et al., 2007; Wilkie & Whittaker, 2006).

4. If the same author has publications in the same year, each should be marked in lowercase letters, starting with the letter "a";

.... (Adams, 2005a; Adams, 2005b;…).

The list of references should be prepared as follows:

1. Reference list should be listed alphabetically by author surname.

2. The names of the journals in the references should be shortened according to the ISI web of Science and should be written in italics.

3. Type of references should be as follows.

Journal article; Sullivan JC, Sasser JM, Pollock JS, 2007: Sexual dimorphism in oxidant status in spontaneously hypertensive rats. Am J Phsiol Integr Comp Physiol, 292 (1), 64-68.

Book; Cadenas E, Packer L, 2001: Handbook of Antioxidants. 2nd ed., Marcel Dekker Inc., New York, USA.

Chapter in a book: Bahk J, Marth EH (1990). Listeriosis and Listeria monocytogenes In: Foodborne Diseases, Cliver DO (Ed), 248-256, Academic Press, San Diego. Web page: Anonymous (1) http://www.emea.europa.eu, Access date; 01.04.2010.

Thesis: Er A, 2009: Effect of macrolide antibiotics on cytokine levels in endotoxemia. PhD thesis, SU Health Sciences Institute, Konya.

Paper presented at the scientific meeting: Allen WR, Wilsher S, Morris L, Crowhurst JS, Hillyer MH, Neal HN, 2006: Re-establishment of oviducal patency and fertility in infertile mares. In: Proceedings of the Ninth International Symposium on Equine Reproduction, Kerkrade, Holland, pp. 27-28.

Tables and Figures: Each table and figure should be placed on separate pages. It should be numbered according to the order of use, expressed in short titles, and should be cited by giving the table number in the text. Table titles should be written in the writing language of the article in the upper part of the table. Abbreviations and necessary explanations used in the table should be given under the table. Figure titles should be written at the bottom of the figure in the writing language of the article.

HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

TELİF HAKKI DEVİR FORMU

Harran Üniversitesi

Veteriner Fakültesi Dergisi Editörlüğüne

Biz aşağıda adı, soyadı ve imzaları bulunan yazarlar, tarafımızdan yazılmış,

.....

.....

İsimli makalenin içeriği, sonuçları ve yorumları konusunda, Harran Üniversitesi Veteriner Fakültesi Dergisi'nin hiçbir sorumluluk taşımadığını kabul ederiz. Sunduğumuz makalenin orijinal olduğunu, herhangi bir başka dergiye yayınlanmak üzere gönderilmediğini, daha önce yayınlanmadığını beyan ederiz. Makalenin telif hakkından feragat etmeyi kabul ederek sorumluluğu üstlenir ve imza ederiz. Makalenin telif hakkı Harran Üniversitesi Veteriner Fakültesi Dergisi'ne devredilerek yayınlanması konusunda yetkili kılınmıştır.

Bununla birlikte yazarların aşağıdaki hakları saklıdır:

1. Telif Hakkı dışında kalan patent v.b. bütün tescil edilmiş haklar.
2. Yazarın gelecekteki kitaplar ve dersler gibi çalışmalarında; makalenin tümü ya da bir bölümünü ücret ödemeksizin kullanma hakkı.
3. Makaleyi ticari amaçlarla kullanmamak koşulu ile çoğaltma hakkı.

Yazarın Adı ve Soyadı	Tarih	İmza
……	……	…….
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