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Research Article

The effects of ethanol extract of *Punica granatum L. peel* on the testis damage induced by diabetes in rats

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This study was presented as a poster at the Experimental Biology Congress, San Diego (2018).

ABSTRACT

The aim of this study was to determine the effect of ethanol extract of Punica granatum L. Peel on biochemical and histopathological changes in blood and testicular tissue in rats with experimental diabetes. A total of twenty-eight male Sprague-Dawley rats, 7 rats in each group, were used in this study. Group 1; peros physiological saline to the rat, Group 2; STZ 60 mg/kg/IP single dose, Group 3; PGE 10 mg/kg/20days/peros, Group 4; STZ as 60 mg/kg/IP + PGE 10 mg/kg/20days/peros. After the end of the experimental procedure, the rats were sacrificed, blood and testicular tissues were taken, and biochemical and histopathological examinations were performed. The administration of PGE was shown that the activities of CAT, SOD, GPx and GSH increased and the levels of MDA decreased in diabetic rats. Compared to the diabetic and control groups, the treatment group decreased the LPO level and improved the antioxidant activity in plasma and testicular tissues. Histopathological examination of testicular tissues in the group 2 is revealed edema in the intertubular spaces, thinning of the tubule walls due to diminished spermatocytes in the walls of the seminiferous tubules and severe degenerative and necrotic changes in spermatocytes. These changes were found to be very mild in group 4. According to immunohistochemical findings, in group 2, caspase 3 expression was intensely expressed in spermatocytes. As a result, it was observed that Punica gra-natum L. bark extract strengthened antioxidant defense and reduced oxidative stress in diabetic rats.

INTRODUCTION

Diabetes mellitus is one of the metabolic diseases that cause functional disorders in many systems, including male infertility (Akhtar et al., 2015). In rats, diabetes causes a decrease in testicular weight, sperm count, total motility and testosterone levels, and increases apoptosis (Cai et al., 2000; Scarano et al., 2006). Diabetes mellitus causes an increase in the level of oxidative stress in tissues and decreases in the antioxidant defense system (Baynes and Thorpe, 1999).

Streptozotocin (STZ), which is used to cause diabetes in rat, is an alkylating chemotherapeutic including a nitrosurea group (Mythili et al., 2004). Further, after STZ enters the beta cells, it is converted into glucose and methylnitrosourea. Because it has an alkylating structure, changes occur in biological mac-romolecules and DNA is fragmented. As a result, beta cells are deformed and a model of insulin-dependent diabetes is formed. By disrupting the mitochondrial DNA of beta cells, it has been shown that insulin release is inhibited by STZ (Len-zer, 2008).

Pomegranate (Punica granatum) is widely used in medicine(Longtin, 2003). Punicalagins, ellagic acid, anthocyanins, flavonoids and a wide variety of antioxidant phenolic compounds are found in pomegranate (Henning et al., 2019). There are approximately 48 phenolic compounds with antioxidant properties in the pomegranate peel(Benzie and Wachtel-Galor, 2011). In addition, pomegranate peel contains punicalagin and ellagitanens such as punicalagin (Newman and Lansky, 2007). Compounds with this antioxidant property are generally found in the pericarp and mesocarp layers of the pomegranate peel (Fischer et al., 2011) and have strong antioxidant activity, anti-mutagenic, anticancer, anti-inflammatory and anti-diabetic effects (Akhtar et al., 2015).

In the light of all these data, in this study, it was aimed to determine the effect of ethanol extract of Punica granatum L. bark on the pathological and biochemical changes in experimental diabetic rats.

MATERIAL and METHODS

Plant Material and Extraction Procedure

The pomegranate peel extract was made with the modification of the method described by Tayel et al.(Tayel and El Tras, 2010). Commercially available pomegranates were separated

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from the seeds and the peels were dried in the shade and were crushed. 100 grams of pomegranate peel was taken and placed in a 1 liter flask, 500 ml of ethanol was added to it and left for 48 hours. The resulting mixture was filtered and coarse particles were separated. Then, this mixture was separated from the organic solvent part with the help of evaporator and the extract was obtained. The extract was stored at +4ºC until the experimental stage to be used in the study.

Experimental Design

In this study, 28 male Sprague-Dawley rats weighing 200-250 grams, 5-6 weeks old, were used as the control and experimental groups. Rats were fed with standard water and pellet feeds. It was kept at 21 °C (±2) in the 12 hour light / 12 hour dark cycle.

Group I (n:7); Physiological saline via oral gavage to the rat.

Group II (n:7); STZ (60 mg / kg) via intraperitoneal injection(IP).

Group III (n:7); Punica granatum L. Extract(PGE) was administered to rats (10 mg/ kg) peros.

Group IV (n:7); STZ (60 mg / kg) via IP+PGE (10mg/ kg) peros.

STZ was dissolved in cold citrate buffer and administered IP after 18 hours of fasting to induce diabetes. The blood glucose level was measured with a glucometer in the rats in which diabetes was desired. Rats with a glucose level of 250 mg/dl and above were evaluated as diabetic. PGE was given to the rats in the experimental groups by oral gavage for 20 days. At the end of the 20th day, blood and tissue samples were taken by performing decapitation under anesthesia. Blood samples taken into anticoagulant tubes were centrifuged at 3000 rpm at +4°C for 10 minutes, the plasma part was removed and stored at -20°C until analysis. Testicles of rats were removed and cleaned from surrounding tissues. The testicular tissues obtained were washed with 0.15 M potassium chloride (KCl) at +4 °C and dried. Then, the tissues were homogenized with a homogenizer in 0.15 M KCI solution at 16000 rpm for 3 minutes. Homogenization was carried out in an ice bucket. The homogenate was centrifuged at 5000 g for 1 hour (at + 4 ° C) then GSH, MDA, CAT, GPx and SOD levels were measured from the supernatant with the help of Biotek ELISA Reader.

Measurement of Oxidative Stress

Testicular tissue CAT activity was measured by the method described by Goth (1991). Testicular tissue MDA level was measured by the method specified by Placer et al. (1966), SOD activity by Sun et al. (1988), and GPx level by Matkovics et al. (1988).Tissue extraction and analysis of GSH was done according to the method of Ball (1966), Fernandez and Videla(1981).

Plasma MDA levels were measure by the method of Yoshioka et al.(1979) and GSH, CAT, SOD, GPx levels were determined according to the method of Tietze(1969), Goth(1991), Sun et al.(1988), Matkovics et al.(1988) respectively, using Biotek ELISA Reader.

Immunohistocemical and in situ Hybridisatiaon

Testis tissues were taken into 10% buffered formalin solution. The samples were embedded in paraffin blocks and 4μm thick sections were stained with hematoxylin-eosin using a microtome and examined with a light microscope for immunohistochemical examination. Sections were classified as absent (-), mild (+), moderate (++), and severe (+++) according to the degree of lesion.

For immunoperoxidase analysis, all sections taken from adhesive (poly-L-Lysine) wares were passed through xylol and alcohol series, deparaffinized and dehydrated. Samples were washed in distilled water for 5 minutes. Sections were heated in citrate buffer (pH 6.1) 4 times in a microwave device for 5 minutes to ensure antigen recovery in the nucleus. Samples removed from the microwave were incubated for 30 minutes at room temperature. Then the samples were washed with distilled water, the sections were dried and drawn with a special glass pen. Endogenous peroxidase was washed with phosphate buffer solution (PBS) for 5 minutes and inactivated by holding in 3% H_2O_2 for 10 min. After washing the sections in PBS samples were left to incubate for 5 minutes with a Protein block compatible with all primary and secondary antibodies to prevent nonspecific background staining. Primary antibodies (caspase 3) were dropped without washing after the excess block solution remaining on the tissue sections at the end of the incubation was poured. It was kept at room temperature for 1 hour or at +4 ^{0}C for 1 night in accordance with the primary antibody. It was washed with PBS 2 times for 5 minutes and incubated with biotinized secondary antibody about 30 minutes at room temperature. The sections washed again with PBS were kept in streptavidinperoxidase for 30 minutes and then washed in the same way with PBS. After washing, AEC (3-amino-9-ethyl carbazole) chromogen was dropped to the sections and kept for 5-10 minutes depending on the chromogen retrieval. For the floor staining, it was kept in Mayer's hematoxylin for 1-2 minutes and then washed in tap water. It was coverslipped using a water-based adhesive and examined with a light microscope (Leica DM 1000). Incubation time in the other stages with primary antibodies, whether antigen retrival or enzyme will be applied, and dilution rates of primary antibodies may vary depending on the commercial kit used. Sections were evaluated as none (-), mild (+), moderate (++) and severe (+++) according to immune positives.

Statical Analysis

The data obtained in the study were analyzed in the SPSS (V13) program. Kruskal-Wallis test was used to determine the difference between groups. The difference between the two groups was determined using the Mann-Whitney U test.

RESULTS

When testicular and plasma biochemical parameters are evaluated (Table 1, 2), The MDA levels in the Group2 were found to be higher compared to the other experimental groups. The levels of GSH, CAT and SOD were lower in the group 2 in comparison the other groups. While a clearly positive effect was observed in the group 3 in terms of the evaluated parame-

ters, the parameters detected in the group 4 confirm the protective efficacy of *Punica granatum L. peel extract* against STZ.

Table 1. Testicular biochemical parameters (Mean ± SEM)

Groups	GSH (mmol/g)	MDA (nmol/g)	CAT (kU/g)	GPx (U/mg)	SOD (EU/mg)
Group I	0.62±0.00^{c}	36.01±0.26^{a}	239.90±1.14^{b}	0.05±0.00^{b}	5.12±0.08^{c}
Group II	0.33±0.00^{a}	56.63±0.44^{c}	213.07±0.86^{a}	0.03±0.00^{a}	3.41±0.08^{a}
Group III	0.64±0.00^{c}	34.42±0.69^{a}	248.34±2.05^{c}	0.06±0.00^{c}	7.36±0.09^{d}
Group IV	0.54±0.01^{b}	38.53±0.63^{b}	239.31±1.46^{b}	0.04±0.00^{a}	4.69±0.06^{b}

a-c The values represented by different letters within the same row are significantly different from each other, **P<0.05

Table 2. Plasma biochemical parameters (Mean ± SEM)

Groups	GSH (mmol/g)	MDA (nmol/g)	CAT (kU/g)	GPx (U/mg)	SOD (EU/mg)
Group I	3.13±0.00^{b}	27.07±0.14^{b}	256.79±0.50^{c}	0.23±0.01^{a}	13.90±0.08b
Group II	2.66±0.03^{a}	37.10±0.18^{c}	138.48±0.42^{a}	0.21±0.00^{a}	11.79±0.16a
Group III	3.18±0.06^{b}	25.72±0.05^{a}	259.15±1.95^{c}	0.25±0.00^{b}	15.07±0.13c
Group IV	3.07±0.06^{b}	27.17±0.39^{b}	243.91±1.78^{b}	0.22±0.00^{a}	13.72±0.11b

a-c The values represented by different letters within the same row are significantly different from each other, **P<0.05

Group 1: Normal testicular histological structure is seen in rats. (Figure1- A).

Group 2: Edema in intertubular spaces and dilated and hyperemic vessels were observed in testicular tissues of rats. It was observed that the walls of the tubules became thinner due to the reduction of spermatocytes in the walls of the seminiferous tubules. In addition, severe degenerative and necrotic changes were detected in spermatocytes. Very few spermatozoa were found in the lumen of the tubules, and some were absent. (Figure1- B).

Group 3: It was observed that both the wall and interstitial spaces of the seminiferous tubules were in normal histological structure in the testicular tissues of the rats. (Figure1- C).

Group 4: Mildly degenerative spermatocytes were found in the tubules of the testicular tissues of the rats in this group, but no necrotic spermatocytes were found. It was determined that there were moderate levels of spermatozoon in the tubulus lumens (Figure1- D).

In addition, ın rats with diabetes, thinning of the tubular wall, degeneration of spermatocytes, necrotic spermatocytes and edema in intertubular spaces were observed. however, it was determined microscopically that orally administered PGE caused a decrease in these changes (Table 3).

Table 3: Histopathological and immunohistochemical scores of testicular tissues.

Groups	Thinning of tubulus wall	Degenerative spermatocytes	Necrotic spermatocytes	Edema in intertubular spaces	Caspase 3
Group I	-	-	-	-	-
Group II	+++	+++	++	+++	+++
Group III	-	-	-	-	-
Group IV	-	+	-	-	++

It was observed that Caspase 3, which was used to determine apoptosis, was expressed at a very low level in the testes of rats in the control and groups 3. This situation was evaluated as negative (-) (Figure 2, A-C). In the group 2, a large number of spermatocytes were found to be positive (+++) (Figure 2, B). Mild positive (+) cells were found in the testicles of rats in the group 4 (Figure 2, D). When the groups 2 and 4 were compared in terms of the number of caspase positive cells, a statistically significant difference was found (p <0.05).

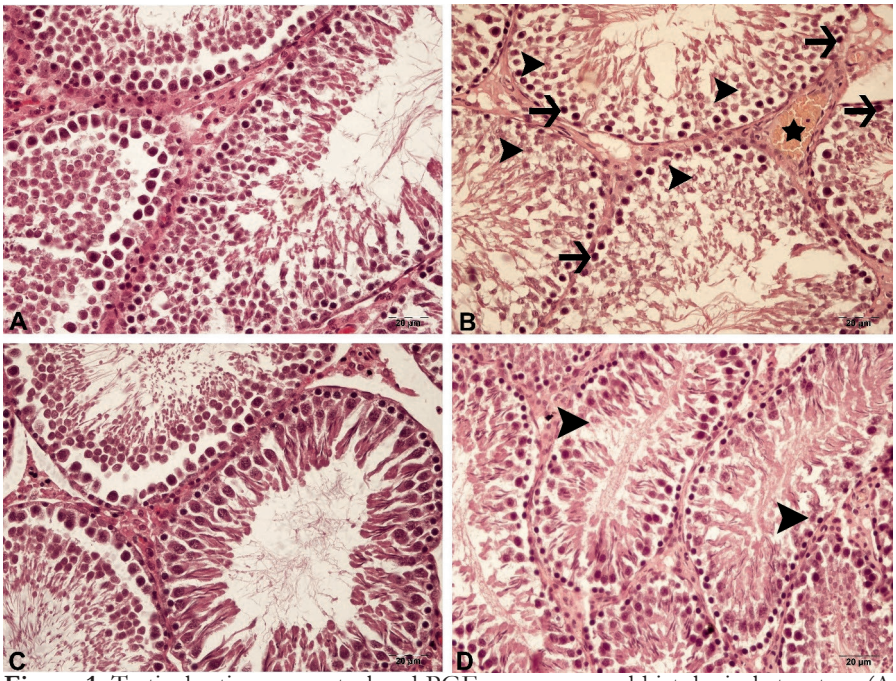

Figure 1. Testicular tissues, control and PGE group, normal histological structure (A and C). Severe necrotic-degenerative spermatocytes in tubuli (arrows-arrowheads), thinning of tubulus wall, dilated and hyperemic vessels in intertubular spaces (star), edema in intertubular spaces (B). Mild degenerative spermatocytes in tubuli (arrowheads), moderate spermatozoon in tubulus lumens (D), H&E, Bar: 20µm.

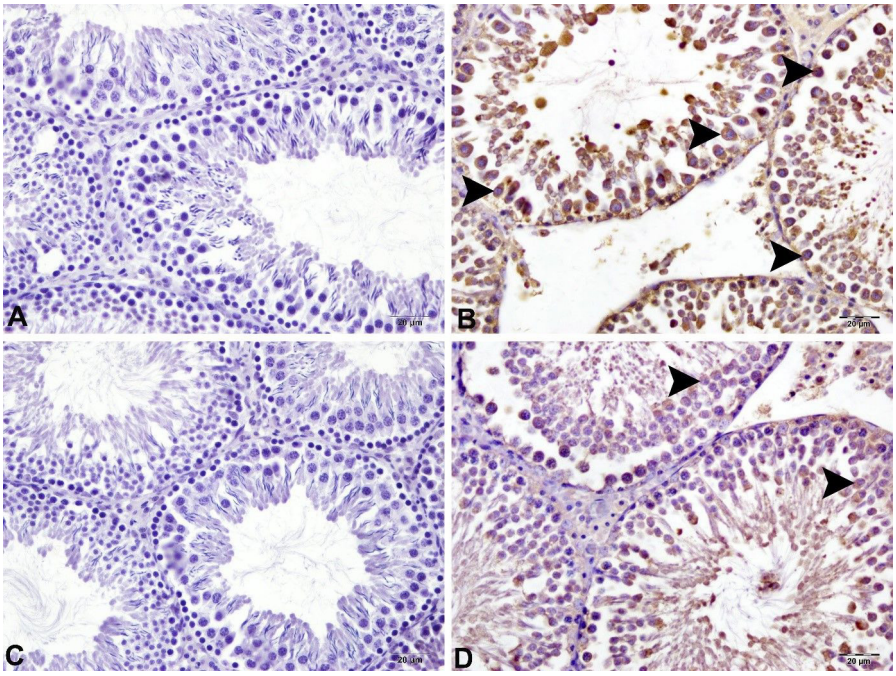

Figure 2. Testicular tissues, control and PGE group, caspase 3 negative (A and C). Severe caspase 3 positive in spermatocytes (arrowheads) (B). Testicular tissues, mild caspase 3 positive in very few spermatocytes (arrowheads) (D), IHC-P, Bar: 20µm.

DISCUSSION

Diabetes Mellitus is a chronic disease characterized by hyperglycemia and significantly affects many functions of the body. It is also important in that it causes a decrease in fertility(Khaneshi et al., 2013). STZ and alloxane are the agents most commonly used to induce experimental diabetes. Diabetes can be caused by intraperitoneal, subcutaneous, intravenous, parenteral administration of these agents. These agents cause hypoinsulinemic and hyperglycemic state by destroying beta cells in Langerhans islets in the pancreas(Erbaş, 2015).

In the present study, it was determined that oxidative stress products increased and antioxidant enzymes decreased in STZ applied groups. In histopathological examination of testicular tissues, edema in the intertubular spaces, thinning of the tubular walls due to the decrease of spermatocytes in the walls of the seminiferous tubules, severe degenerative and necrotic changes in the spermatocytes were detected. Ibrahim (2018) stated that diabetes related to STZ, similar to our study, causes a disorder in the reproductive system in male rats. This situation is thought to be related to the fact that STZ increases oxidative stress and consequently leads to a decrease in sperm quality and causes histopathological changes in the testis.

It has been reported that a new ellagitannin and punigluconin containing a gluconic acid were found in the fresh body peels of P. Granatum (Tanaka et al., 1986). So, when evaluated in terms of antioxidative properties, because of the content of P. Granatum, it was observed in the current study that Punica granatum L peel extract increased antioxidant activity and decreased malondialdehyde levels in testicular tissue and plasma of diabetic rats.

Studies have reported that diabetes increases oxidative stress due to hyperglycemia, causes damage to the seminiferous tubules and drives spermatocytes to apoptosis(Khaneshi et al., 2013). In our study which supports the findings; thinning of tubule walls, severe degenerative and necrotic changes in spermatocytes were found to be very mild in the group 4. According to the immunohistochemical findings, caspase 3 expression, which was performed to detect apoptosis, was severely expressed in spermatocytes. On the other hand, it was observed to be very mildly expressed in the group 4.

In a study by Mahmoud and Mahmoud(2017), it was determined that pomegranate peel extract provided protection against damage to the tissues of rats, which were evaluated differently from our study in the diabetes table created with STZ. In addition, in another study, it was determined that pomegranate peel extract was effective against oxidative stress in rats with oxidative stress(Doostan et al., 2017).

In addition, according to the findings of the study by Dkhil et al. (2013), it was observed that the application of Methanolic Bark Extract and Pomegranate (Punica granatum L.) juice decreased oxidative stress in testicular tissues of rats and increased antioxidant activity. Also, in view of the application of Pomegranate peel extract, results showed an improvement of morphological condition of seminiferous tubules in adult wistar rats(Boroujeni et al., 2017). And, in another study, Minisy et al.(2020) have shown pomegranate seeds extract to be effective against testicular toxicity in experimental rats. Again, in this sense, a study on rabbits is also remarkab-le. Bakeer et al.(1988) found that pomegranate peels stimulate spermatogenesis and increase fertility in rabbits. These situations were consistent with the results we obtained.

The mechanism of action of free radicals resulting in lipid peroxidation (LPO) is as follows: Free radicals are atoms or molecules that contain one or more unpaired electrons, and they react rapidly with other molecules to share these electrons. Since one electron of molecules that react with radicals decreases, they become reactive and this reaction continues in a chain. Abundant free radicals are produced in mitochondrial, endoplasmic and nuclear electron transport systems (cytochrome P-450), peroxisomes, and during normal metabolic events such as phagocytosis of monocytes and neutrophils. Many defense mechanisms have been developed in the body to prevent the formation of these radicals and the damage they may cause. If these radicals exceed the capacity of the defense mechanisms, they cause damage to important components of cells such as lipids, proteins, DNA, carbohydrates and enzymes. Lipids are the structures most susceptible to free radical damage. Free radicals easily react with unsaturated bonds in fatty acids and cause peroxidation of lipids(Aydilek and Aksakal, 2003).

In general, antioxidant substances prevent the stealing hydrogen by free radicals from tissues. They provide this situation by directly supplying the hydrogen needed by free radicals. In other words, antioxidant substances saturate free radicals at the beginning and prevent their continuous activities. However, if the formation of free radicals continues due to light and metals, the antioxidant substance is consumed. If necessary precautions are taken and sufficient amount of antioxidant material is present, free radicals will be stopped before and antioxidant effect will continue for a long time(Çakmak, 2003). The effects of antioxidants against free oxygen radicals are as follows; stopping the initiation of the chain reaction, breaking the radical chain reaction that started, preventing the radical formation to start. In addition, they disrupt the structure of peroxides and reduce the local oxygen density(Cheeseman and Slater, 1993). By the way, the most important antioxidant enzymes; SOD, which converts superoxide anion to H_2O_2, GPx, which detoxifies organic peroxides and catalase that H_2O_2, reduces to H_2O(Marti et al., 2008). When the effectiveness of antioxidant enzymes mentioned in the findings section of our study is interpreted in terms of literature information, the results are significant.

In the light of the literature, the results of many different studies related to the positive effect of pomegranate on reproductive parameters in lab animals support our findings(Lydia, 2019).

CONCLUSION

In conclusion, it was concluded that lipid peroxidation of testicular damage occurs in the case of diabetes induced by STZ and pomegranate peel extract (PGE 10 mg/kg/20days/po) prevents this damage.

DECLARATIONS

Ethics Approval

The rats used in the study were obtained from Atatürk University Experimental Application and Research Center (ATADEM). Permission was obtained from the Local Ethics Committee of Ataturk University (75296309-050.01.04-E.2000065644/4).

Conflict of Interest

The authors declare that they have no known competing fi-

nancial interests or personal relationships that could have appeared to influence the work reported in this paper.

Consent for Publication

The authors have given the consent for publication to the journal.

Author contribution

Idea, concept and design: ADO, BAY

Data collection and formal analysis: ADO, BAY, SY

Drafting of the manuscript: ADO, SAA

Critical review, writing and editing SAA

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Not applicable

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Research Article

A stereological study on determination of ventricular wall volume of the heart in female and male quails

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ABSTRACT

In this study, ventricular wall volume of female and male quails was investigated stereologically. Six females and six males quails were used in this study. All of the animals were perfused. After the perfusion, the quails were kept in 10% formaldehyde solution. Afterwards, chests of quails were cut and their hearts were resected. Ventricles of the hearts were separated. Specific ratio of tissue samples was obtained from each ventricle. The 5-µm thick samples were cut by using a microtome. Sequentially, 10 sections were obtained. These sections were stained by hematoxylin eosin and photographed. Volumes of wall of the ventricles were estimated by using the Cavalieri's Principle. In this study, the volume values of ventricular walls of female and male quails were compared. Some differences were found between these values. The volume values of ventricular walls of six female quails were compared with each other. While the lowest volume value was 0.398 cm^3, the highest volume value was 0.612 cm^3. The volume values of the male quails were between 0.438 cm^3-0.817 cm^3. It was found that volume values of ventricular walls of male quails were higher than volume values of ventricular walls of female quails. As a result, although there was a specific distinction between volume values of ventricular walls of female and male quails. There was no difference between statistic values ($P>0.05$). It was thought that this study will be guiding for other related studies.

INTRODUCTION

In birds, the circulatory system carries oxygen, nutrients and hormones necessary for the continuation of life, and ensures that the wastes of metabolism and carbon dioxide are expelled from the cells (Dursun, 2002). The heart, the center of the circulatory system, is a hollow muscular organ (Dursun, 1994). Although the position of the heart varies according to the animal species, it is surrounded by the pericardium in the caudoventral direction in the cranial region of the thoracoabdominal cavity in birds (Dursun, 2002). In birds, the heart consists of the right atrium, left atrium, right ventricle and left ventricle, as in mammals (Dursun, 1994; Dursun, 2002). The right ventricle reaches only 2/3 of the heart, and the apex of the heart is formed by the left ventricle. It has been noted that the left ventricle is thick-walled and cone-shaped and appears circular in cross-section. (Dursun, 2002). Various studies have been conducted to accurately and clearly determine the volume of the heart. Many studies on experimental animals and humans have revealed right ventricular and left ventricular volumes by various methods (Mahoney et al., 1987; Aebischer and Czedledy, 1989; Eishstacdt et al., 1992; Heusch, 1999; Cui et al., 2004). Methods that can be used to monitor the heart ventricles and calculate their volume are used in the diagnosis and treatment of heart diseases. It has been suggested that some heart diseases may lead to deformations in the ventricles and deviations in the ventricular volume (Noerdegraaf et al., 1997). In a study conducted on Kivircik sheep and hair goats, the volumes of right ventricle side wall, left ventricle side wall, right ventricle cavity, left ventricle cavity and interventricular septum were calculated (Ince and Kahvecioglu, 2010). In another study on dogs, measurements obtained with the principle of fluid displacement in calculating the volume of the right ventricle were compared with echocardiographic measurements and a positive ratio was found between them (Aebischer and Czedledy, 1989). The volume values were calculated using balloons placed in the heart cavities by another method (Sapin et al., 1993; Siu et al., 1993). It was determined that the methods used to evaluate the heart volume were not sufficient to preserve the anatomical structure of the heart.

The volumes of objects with regular or symmetrical shapes are calculated using the following mathematical formula; $V=t\times a$. In this equation, V is the volume, t is the height of the object, and a is the base area of the object (Sahin et al., 2003a; Sahin et al., 2003b). The Cavalieri's Principle is used to

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calculate the volumes of objects with an irregular shape (Odaci et al., 2003). To calculate the volume ratio, the formula Vv.(Y.ref)=Pp(Y.ref)=P(Y)/P(ref) is used according to the Cavalieri Principle (Howard and Reed, 1998).

It is known that it is possible to obtain the most accurate quantitative analyzes with stereological methods used to calculate the volumes of three-dimensional objects (Howard and Reed, 1998; Glaser and Glaser, 2000; Bertram, 2001; Odaci et al., 2003; Sahin et al., 2003a; Sahin et al., 2003b).

Recently, a special importance has been given to poultry farming in Türkiye. Considering this importance, it is aimed to calculate the volume of a quail heart. It is believed that this study will shed light on all morphological and anatomical studies on the heart of birds, as well as stereological studies.

MATERIAL and METHODS

Animals

In this study, 6 adult female healthy quails and 6 adult male healthy quail (*Coturnix coturnix japonica*) two months old and weighing 180±5 g were used. 10% buffered formaldehyde solution was applied by intracardiac perfusion technique (Romeis, 2001). The quails anesthetized with ketamine hydrochloride (50mg/kg i.m) injection were kept in 10% formaldehyde solution pool for one week (Aslanbey et al., 1987).

Resection of the heart

The chest cavity of quails was opened at the level of the sternum to resect their hearts. The heart, aorta, and pulmonary vein were resected in the thoracic cavity.

Sampling type

Before starting the study, a pilot study was specifically planned. With this planned pilot study, it was aimed to determine the number of animals, the number of sections and sampling to be used in the study. Studies have shown that in a stereological study at least five animals in a group must be used to achieve an error coefficient of approximately 0.05 (Weibel, 1980; Cruz- Orive and Weibel, 1990). Calculation of the error coefficient was based on the square root of the total variant/total number of points. For this cross-check method, it was determined that the number of individuals in the sample group was sufficient by using the SHTEREOM I package program (Gundersen and Jensen, 1987).

Five quails were used for the pilot study. Tissues were dissected and embedded in paraffin after tissue follow-up. Random 5 µm thick transversal sections were taken from the quail hearts using a Rotary microtome (Leica RM 2135, Nussloch, Germany) from the beginning of the tissue to the end and transferred to slides after the procedure. After the sections were deparaffinized, they were stained with hematoxylin-eosin and covered with a lamella (Luna, 1968) (Figures 1, 2, 3, 4). The sections were sampled at the ratio of 1/40 as 8-10 sections for each animal. Systematic random sampling was preferred, and any sections after the first 40 sections were taken and the next 40 sections continued to be taken. It was determined that

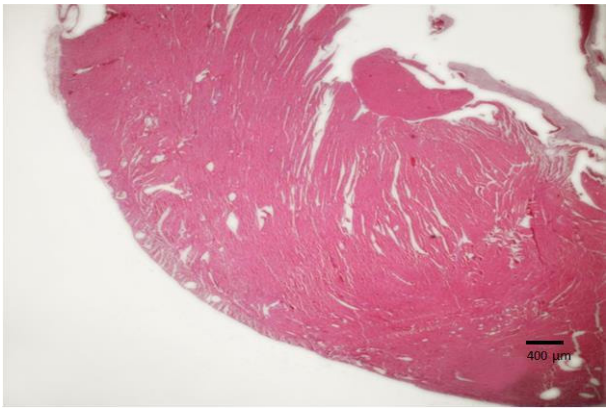

Figure 1. The ventricular wall of the quail X1.25 magnification (Hematoxylin eosin).

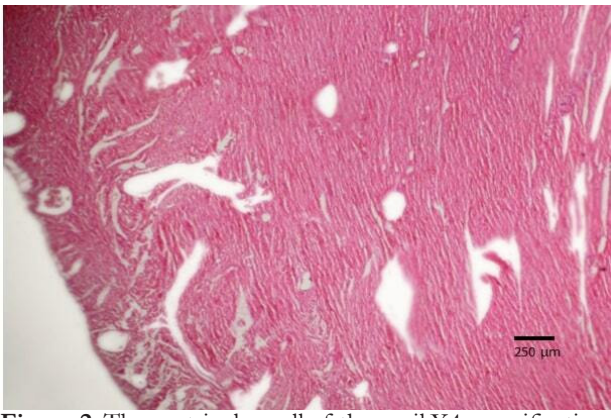

Figure 2. The ventricular wall of the quail X4 magnification (Hematoxylin eosin).

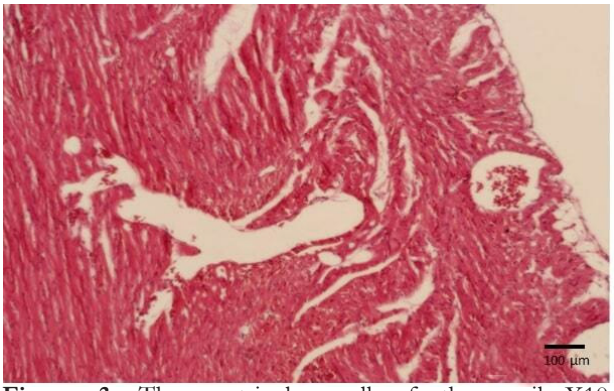

Figure 3. The ventricular wall of the quail X10 magnification (Hematoxylin eosin).

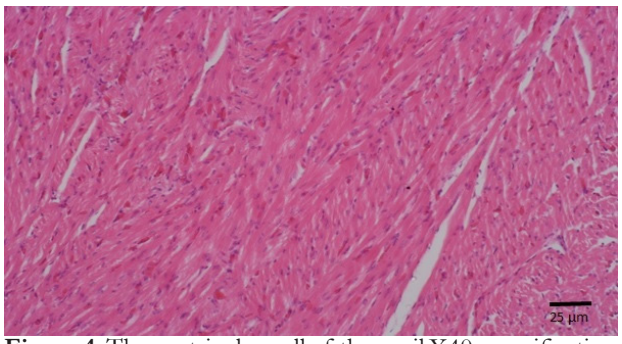

Figure 4. The ventricular wall of the quail X40 magnification (Hematoxylin eosin).

10 sections should be taken according to the values obtained from the pilot study. The study was carried out according to the values determined in the pilot study.

Image Analysis

Because of the quail heart has a medium-sized structure, stereological stepping was necessary. Images were obtained with objective x10 magnification using a motorized table with stereological stepping method. Approximately 30-35 images were obtained for each section. Using the point grid, the area was calculated at objective x10 magnification, and then the volume was calculated. SHTEREOM I package program was used for this process. The calculation basis of the program is based on the Cavalieri's Principle (Odaci et al., 2003). The entire ventricular volume of the heart was determined as a whole. Right ventricular and left ventricular volumes and spaces were also counted. Only the ventricular wall volume was calculated, since results close to the truth could not be obtained. This is because the shrinkage rate cannot be calculated precisely and accurately due to the water lost during tissue processing and other processes.

Calculation was made with the number of points because the numerical ratio of the points is considered valid instead of was taken as a basis.

Volume of the ventricular wall = volume rate × reference volume of the ventricular wall

Reference volume of ventricular wall = distance of the section range × real area of the point × number of points

The ventricular wall volume of the heart was measured according to Archimedes' fluid displacement principle. 10 ml of distilled water was placed in the graduated cylinder. After the hearts were thrown into the water in the graduated cylinder, the amount of water they overflowed was calculated. The volume values of the ventricular wall of male and female quails were obtained according to Archimedes' fluid displacement principle.

RESULTS

The ventricular wall total volume values of six quails in the same age group from female animals were B1=0.516 cm^3, B2=0.589 cm^3, B3=0.556 cm^3, B4=0.485 cm^3, B5=0.398 cm^3 and B6=0.612 cm^3, respectively. from the first to the last. In this study, the highest volume value among female animals was calculated as 0.612 cm^3 (Table 1) (Figure 5).

Table 1. The volume values of ventricular wall of quails (Q) (cm3).

Quail (Q)	Female	Male
Q1	0.516	0.438
Q2	0.589	0.617
Q3	0.556	0.598
Q4	0.485	0.817
Q5	0.398	0.590
Q6	0.612	0.610
Means	0.526	0.611

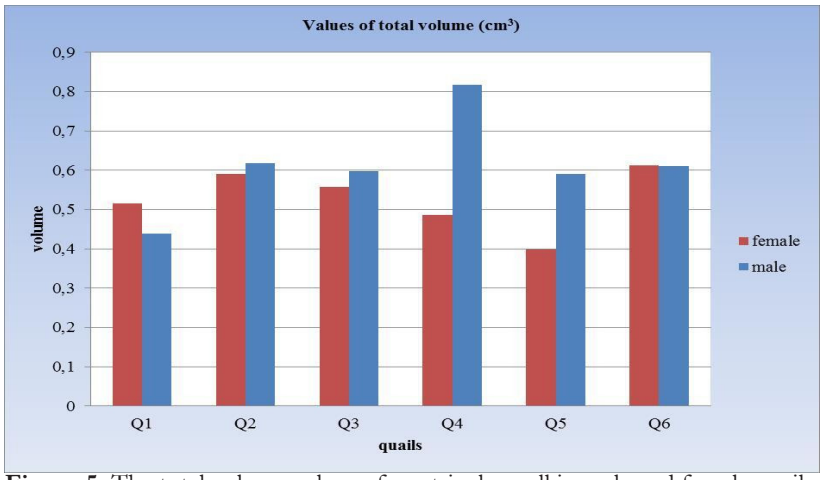

Figure 5. The total volume values of ventricular wall in male and female quails.

the volume in volume calculations (Howard and Reed, 1998; Howard and Reed, 2005). In order to avoid unnecessary point counts in all tissues, counting the points at regular intervals

The ventricular wall total volume values of male quails in the same age group were calculated as B1=0.438 cm^3, B2=0.617 cm^3, B3=0.598 cm^3, B4=0.817 cm^3, B5=0.590 cm^3 and

B6=0.610 cm^3. In the evaluation made among male animals, it was seen that the highest volume value belonged to the B4 coded animal. The highest value following this was observed as 0.81 cm^3. Although the volume values in male animals seem close to each other, the difference between the values was not remarkable since there was no statistical difference. When the volume values of male and female animals were compared, it was seen that the ventricular wall volume values were higher in favor of male quails (Table 1) (Figure 5). While the coefficient of error (CE) value obtained in the study was 0.04 in female quails, it was 0.037 in male quails. In this case, since the error rate coefficient CE<0.05 is a reasonable value, it has been determined that the results are reliable (Table 2).

quails was 725 points. In male quails, this value was found to vary between 604 points and 1126 points. It was determined that the average number of points per area in male quails was 843 points (Table 3). It was noted that the average number of points per area in male quails was higher than in female quails. Obtaining these values was an important result for the present study.

Based on Archimedes' principle of fluid displacement, the volume of the ventricular wall of the heart was measured. The ventricles were thrown into the water as a whole according to the Archimedes' principle. The ventricular cavity was also included in the calculation of the volume values of the

Table 2. The values of Coefficient of Error (CE)

Quail (Q)	Female	Male
Q1	0.0424	0.0422
Q2	0.0366	0.0369
Q3	0.0371	0.0364
Q4	0.0449	0.0366
Q5	0.0451	0.0371
Q6	0.0366	0.0378
Means	0.0408	0.0378

Table 3. The number of points per area (Noise (N)).

Quail (Q)	Female	Male
Q1	711	604
Q2	812	851
Q3	767	824
Q4	669	1126
Q5	549	814
Q6	844	841
Means	725	843

Table 4. The volume values of ventricular wall of quails by Archimed Principle and stereological method.

	Female		Male	
Quail (Q)	**Archimedes (ml)**	**Stereological (cm^3)**	**Archimedes (ml)**	**Stereological (cm^3)**
Q1	1.5	0.516	2.0	0.438
Q2	1.0	0.589	2.0	0.617
Q3	1.0	0.556	1.5	0.598
Q4	1.5	0.485	1.0	0.817
Q5	0.5	0.398	1.5	0.590
Q6	1.5	0.612	2.0	0.610

Based on the number of points per area, it was observed that this value varied between 549 points and 844 points in female quails. The average number of points per area in female

ventricles thrown into the water. Unlike Archimedes' principle, only the volume of the ventricular wall was calculated using the stereological method. The volume of the ventricular cavity

was not included in the measurement in the stereological study. Therefore, a difference was determined between the volume values, which were obtained by the stereological methods, and the volume values, which were obtained by the Archimedes' principle (Table 4). After the ventricles were placed in water, their values and the difference between them were obtained.

Statistical Analysis

SPSS (SPSS for Windows) package program was used in the statistical analysis of the data. First of all, normality test was performed to determine whether the data were normally distributed. According to the normality test, it was determined that the data were normally distributed with 95% confidence. Then, independent samples t-test was performed to reveal the difference between genders. As a result, there was no statistically significant difference between the genders in terms of total volume values (P> 0.05). In addition, it was determined that male animals were only numerically higher than the total volume values of female animals (Table 5).

Table 5. Statistical analysis.

Total Volume Statistics			
Sex	**Number of Animals**	**Mean±Std. Deviation**	**Significant Value**
Male	6	0.612260±0.120821466	0.175
Female	6	0.526592±0.078058562	

DISCUSSION

Although different volume calculation techniques are used in scientific research, it is discussed whether these techniques have superiority to each other (Gundersen, 1986; Gundersen and Jensen, 1987; Howard and Reed, 1998). By means of the stereological methods that provide data and interpretations regarding the real three-dimensional structural properties of two-dimensional cross-section images obtained from histological materials by various methods; morphometric values such as volume, surface area, number and length can be obtained (Weibel, 1980; Unal et al., 2002).

In other words, new approaches called stereological methods are widely used in the morphometric evaluation of biological structures (Gundersen, 1986; Gundersen and Jensen, 1987; Howard and Reed, 1998; Gevrek, 2011). Both the use of systematic random sampling method and the use of certain formulas with proven mathematical values that evaluate the scientific structure increase reliability (Mayhew and Gundersen, 1996; Cruz- Orive, 1997). When a biological construct is analyzed by stereological methods, there is no hesitation about the results (Cruz- Orive and Weibel, 1990).

In many studies, the method used to evaluate the ventricular wall volume and the actual measurement values obtained directly from the heart are needed to determine the accuracy of the measurement values. Thus, either the Archimedes' principle (Lipton et al., 1978) or samples of each heart are created and actual measurements are made on these hearts (Heusch et al., 1999; Cui et al., 2004).

In this study, the ventricular volume of the heart was measured based on Archimedes' fluid displacement principle. In this method, the ventricle wall of the heart was considered as a whole and the ventricles obtained from quails were placed separately in a graduated cylinder filled with distilled water. Then the amount of water remaining in the graduated cylinder was measured. In this way, it was thought that the volume values of the ventricle wall of the heart are very subjective values for us and that there may be errors in the calculations due to the irregular structure of the heart. Thus, as a result of the study, stereological measurement values were taken as basis..

In the study on the stereological evaluation of the heart ventricle in Kivircik sheep and hair goats, the volume of the left ventricle, the right ventricle and the interventricular septum and the volume of the ventricular cavity were calculated (Ince and Kahvecioglu, 2010). However, in this study, it was aimed to calculate the whole ventricular wall volume in quails. The study differs in terms of regions calculated with the study of Ince and Kahvecioglu. In addition, the volume values of the right ventricle and left ventricular cavities were not calculated in this study.

In another study, the effects of prenatally administered diclofenac sodium on the heart tissue of postnatal rats were investigated by stereological and histological methods using the Cavalieri principle (Gevrek, 2011). When the results of this study were evaluated, it was seen that diclofenac sodium given at a dose of 1 mg/kg before birth affected the development of the heart. Although there are similarities in stereological methods and volume calculation methods between this study on the heart of rats and our study, they differ with drug administration.

CONCLUSION

In the present study, the Cavalieri's principle was used to calculate the volume of ventricular walls in the hearts obtained from male and female quails. This study was planned due to the limited number of studies on the morphometry of the heart in birds and the lack of a stereological study on the calculation of the ventricular wall volume values. It was thought that this study would both contribute to the literature and shed light on future studies on anatomy, morphology, and histology of heart as well as stereological studies. Furthermore, it was hoped that

this stereological study will be the basis for the studies on different birds and various systems.

DECLARATIONS

Ethics Approval

Final report of the research project was approved by Van Yuzuncu Yil University Animal Researches. Local Ethical Committee in the session held on 28/06/2018 decision number 2108/06

Conflict of Interest

There is no conflict of interest between authors.

Consent for Publication

Publication permission approved by all authors

Author contribution

Idea, concept and design: GÇ

Data collection and analysis: GÇ, HK, MÇR, MK, VA

Drafting of the manuscript: GÇ, HK, ZS, MÇR

Critical review: GÇ, HK, ZS, MÇR

Data Availability

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Research Article

Outcome of preputioplasty in cats with acquired phimosis: 8 cases

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ABSTRACT

Preputioplasty is a surgical procedure performed to increase the orifice diameter. This study was aimed to describe the clinical outcome of eight feline acquired phimosis that underwent preputioplasty. History, age, breed, body weight, urine and blood analysis, radiographic, ultrasonographic and clinical findings at the time of diagnosis were recorded and collected. Surgical procedures in cats were completed without any complications except for one cat, which had post-operative stricture formation. Normal urination was observed following surgeries in all cases. This cat required perineal urethrostomy surgery. In conclusion, preputioplasty was performed successfully in 7 of 8 cats. The outcome of preputioplasty in cats was good, except for one case that needs a second surgery due to a stricture subsequently formed. Future studies with larger case numbers are needed to confirm our findings.

INTRODUCTION

Phimosis is characterized by the inability of the penis extrusion due to the absence or narrowing of the preputial orifice. It can be congenital or acquired in cats (De Vlaming et al, 2019). The most common causes of acquired phimosis in cats are neoplasia, cellulitis, inflammation, edema or scar tissue that occurs after trauma, sucking by littermates or licking by the dam (De Vlaming et al, 2019; Meilán, 2006). Clinical signs may be asymptomatic or life-threatening, depending on whether the pubertal orifice allows urination or not. Clinical findings in cats with acquired phimosis include stranguria, pollakiuria, preputial swelling, reluctance to mating and secondary bala-noposthitis and ulceration caused by urine accumulation in the preputium cavity (Papazoglou and Kazakos, 2002; Yoon and Jeong, 2013; May and Hauptman, 2013).

The diagnosis of the narrowed preputial orifice can be achieved through the exteriorization of the penis (May and Hauptman, 2013). In veterinary medicine, preputioplasty is a surgical procedure performed to enlarge the orifice diameter in order to treat phimosis. (Papazoglou and Kazakos, 2002; Yoon and Jeong, 2013; May and Hauptman, 2013).

The purpose of this study was to report the clinical outcome of eight cases of feline-acquired phimosis that underwent preputioplasty.

MATERIAL and METHODS

For this retrospective research, the medical records of Atatürk University Veterinary Faculty Animal Hospital were retrieved between December 2018 and December 2022, for client-owned cats that underwent preputioplasty. Data based on history, age, breed, body weight, urine and blood analysis, radiographic, ultrasonographic and clinical findings at the time of diagnosis were collected. Postoperative complications were evaluated as either minor (no need for reoperation) or major complications (requiring reoperation). Follow-up data obtained medical records were noted on the 3rd, 10th, 30th and 60th postoperative days and complications were documented (Figure 1E).

Surgical procedure

After a routine pre-operative physical and complete blood count assessments, medetomidine HCL (80 µ/kg, IM; Domitor®, Zoetis, İstanbul) and tramadol (2 mg/kg, SC; Contramal®, Abdi Ibrahim, Istanbul) were administered for sedation and analgesia, respectively, followed by anesthetic induction with propofol (3 mg/kg, IV; Propofol, Braun-Deutschland). The animals were intubated and anesthesia was maintained with sevoflurane (Sevorane %100, Abbvie, İstanbul) and in 100% oxygen (200 ml/kg/min). At the time of anesthetic induction, cefazolin (20 mg/kg, IV; Maxicilin, Yavuz ilaç, İstanbul) was administered and normal saline was infused intravenously at a

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rate of 10 ml/kg/h during all surgical procedures.

Animals were positioned in sternal (Figure 1A) or dorsal (Figure 1B) recumbency and the preputial region was clipped and prepared for aseptic surgery. The scar tissue was resected by a round-shaped 5 mm incision from the preputial orifice (Figure 1B, C, 2B). The preputial mucosa was opposed to the ipsilateral incised skin edge using simple interrupted sutures of 3-0 polypropylene (Propilen, Dogsan, Germany) (Figure 1D, 2C). Cefazolin (20 mg/kg, IV) and meloxicam (0.05 mg/kg, PO; Boehringer Ingelheim, Istanbul) were administered during the postoperative period for two days. The Elizabethan collar was placed on for 7 days to protect the area from licking.

RESULTS

Animal breeds exposed to acquired phimosis were as follows: one British Shorthair, one Persian cat and six Tabby cats. The median age was 2 (range 12 to 36 months), and the median body weight was 4.3 kg (range 3.9 to 5.3 kg). All cats suffered from stranguria and pollakiuria, while hematuria was observed in one cat. Five cats had considerable preputial swelling due to urine pooling in the prepuce. The duration of clinical symptoms ranged from 1 to 3 months. A urinary catheter was attempted to the bladder through the preputial opening but it was unsuccessful except for one cat. Urinalysis was normal in all cats, however, one cat presented hematuria and urine crystals. In the ultrasonographic examination of the bladder, no

Figure 1. Case 1, A: Preoperative view of the stenotic preputial orifice. B: Intraoperative view of the case following the urethral catheterization. C: Postoperative view of the round-shaped incision technique. The penis can easily extrude from the preputial orifice following the surgery. D: Postoperative 10th day of the case before the suture removal. E: Postoperative 60th day of the case.

abnormalities were detected. In the radiographic examination, the urinary bladder was full and tight.

In all of the cases, preputioplasty was performed. Castration was also performed on all cats with the consent of their owners. Procedures were completed without any complications. Normal urination was provided following surgery in all cases. One cat treated with preputioplasty experienced stricture formation. This cat required perineal urethrostomy surgery one month later.

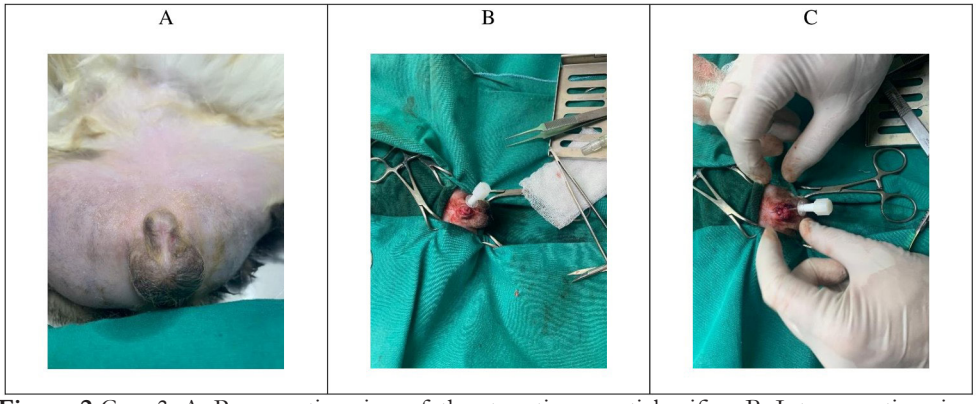

Figure 2 Case 3, A: Preoperative view of the stenotic preputial orifice, B: Intraoperative view of the case following the urethral catheterization C: Postoperative view of the round-shaped incision technique. The penis can easily extrude from the preputial orifice following the surgery.

DISCUSSION

The current study demonstrated that preputioplasty was a rapid and easy procedure for treating acquired phimosis in cats, with no reported postoperative complication except for one cat in the study. Trauma and neoplasia are the main causes leading to acquired phimosis (Meilán, 2006). The reason of phimosis in our cases were unknown, but it may be related to the trauma that causes narrows the preputial orifice. Similar statements have been reported previously (May and Haupt-man, 2013; Bright and Mellanby, 2004).

The prepuce is a skin fold that covers the glans and consists of the external lamina (skin) and internal lamina (mucosa). The preputial orifice is developed at the junctional mucocutaneous tissue which marks the boundary between an internal lamina and an external lamina. The preputial orifice allows the extrusion and contraction of the penis (Yoon and Jeong 2013; Kim et al, 2014). Several methods have been reported for the treatment of phimosis in cats. Reported techniques include a wedge-shaped resection technique, releasing incision with su-tures placed or no sutures placed technique or a round-shaped resection technique (Yoon & Jeong 2013). A round-shaped resection technique (360° incisions around the preputial ori-fice) was applied, the technique is based on the principle of in-creasing the diameter of the orifice by making a wedge-shaped incision at the preputial orifice and removing the scar tissue. Following the incision of the preputial orifice and resection of the tissues, the preputial mucosa is sutured to the ipsilat-eral skin edge (Bright and Mellanby, 2004). Releasing incision with sutures placed or no sutures placed technique includes a full-thickness skin incision on the ventral preputial region without any suture placement. However, in this method, a limited expansion of the preputial orifice is provided only in the ventral region (May and Hauptman, 2013).

In the current study, no postoperative complications were noted in seven cats. However, in one case, recurrence was observed and a perineal urethrostomy was performed. Similarly, a previous study in kittens has also reported good outcomes following preputial preputioplasty (De Vlaming et al, 2019). Perineal urethrostomy may be also used to treat acquired phimosis, but no superiority has been found in preputioplasty techniques. On the contrary, perineal urethrostomy is technically more difficult, costly, more invasive and contains a higher risk of postoperative complications (May and Hauptman, 2013; Catriona, 2007).

The major limitations of the current study are small case numbers and retrospective nature. Future studies with larger case numbers are needed to confirm our findings. After this study, it is still unclear whether other treatment methods have a better prognosis than preputioplasty to treat acquired phimosis in cats. Therefore, this can be considered a limitation of the current study.

CONCLUSION

In conclusion, acquired phimosis occurs in cats, and is easily diagnosed during the physical examination and rapidly treated with preputioplasty. In the current study, preputioplasty was performed successfully in 7 of 8 cats. The outcome of preputioplasty in cats have a good prognosis with minimal postoperative compications.

DECLARATIONS

Ethics Approval

Ethics committee approval is not required for this study.

Conflict of I nterest

The authors declare no conflict of i nterest.

Consent for Publication

Not applicable

Author contribution

The idea, conception of the research and design: SO, LEY

Data collection and analysis: SO, LEY, UE, AG, FT, BG

Drafting of the paper: SO, MGŞ

Critical review: AG, LEY

Data Availability

The data that support the findings of this study are available from the corresponding author upon

reasonable request.

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Not applicable

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Research Article

Determination of gingival temperatures of dogs with healthy gums by means of a thermal camera

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ABSTRACT

In this study, the gingival inflammation degree of dogs was determined according to the Gingival Index (GI). According to this index, it was aimed to determine the free gingiva (FG), attached gingiva (AG) and alveolar mucosa (AM) temperatures of the gingiva of dogs with healthy gums by means of a thermal camera. The material of the study consisted of the gingiva of 140 dogs aged 2 years and older, who were brought to Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Animal Hospital, Surgery Clinic, non-brachiocephalic and without periodontal destruction in their gingiva. Healthy maxillary (n= 427) and mandibular (n= 671) gums in addition maxillary (n=432) and mandibular (n= 463) gums with gingivitis were included in the study. The FG temperature of the buccal surface of the healthy maxillary gingiva was 36.25±1.43°C, the AG temperature was 36.81±1.37°C, and the AM temperature was 37.53±1.30°C. The FG temperature of the buccal surface of the healthy mandibular gingiva was 35.46±1.42°C. In addition the AG temperature was 36.26±1.35°C, and the AM temperature was 37.01±1.22°C. The FG temperature of the maxillary gums with gingivitis was 36.40±1.32°C, the AG temperature was 36.95±1.21°C, and the AM temperature was 37.57±1.21°C. The FG temperature of the mandibular gums with gingivitis was 35.82±1.26°C, the AG temperature was 36.57±1.16°C, and the AM temperature was 37.27±1.10°C. Temperature difference (r) between in maxillary and mandibular FG (r= 0.78°C and p= .000), AG (r= 0.52°C and p= .000) and AM (r= 0.51°C and p= .000) of dogs with healthy gums temperatures were found to be significant. Temperature difference between maxillary and mandibular FG with gingivitis (r= 0.58°C and p= .000), AG (r= 0.38°C and p= .000) and AM (r= 0.29°C and p= .000) were found to be statistically significant. It was concluded that the thermal camera can be an effective diagnostic tool in detecting inflammatory changes in the gingiva.

INTRODUCTION

Healthy gingival tissue is coral pink, with weak and sharp edges (Wiggs & Lobprise, 1997). Edema, hyperemia and bleeding in the gingival margin are the most important findings of gingivitis (Gorrel, 2013). It is the most common oral disease in dogs. It is diagnosed in 80% of dogs over 2 years (Wiggs & Lobprise, 1997). Gingivitis manifests itself with erythema and rounding at the gingival margin. As the inflammation increases, gingival hemorrhages occur and erythema may spread to the entire gingiva (DeBowes, 2010). There is no recession, furcation or tooth mobility in the gingiva (Gorrel, 2013). If the progression of gingivitis is not prevented, infection in the periapical region of the tooth root leads to pulpitis and tooth loss. Endodontic diseases arise as a result of severe periodontal diseases (Reiter & Harvey, 2010). However, early diagnosis and treatment is crucial because this situation causes many important health problems in the animal before tooth loss (Niemiec, 2013).

Subgingival plaque plays a role in the progression of the disease from gingivitis to periodontitis. Bacteria in subgingival plaque secrete metabolic products that initiate inflammation as well as toxins (Harvey & Emily, 1993). In addition, cytotoxins produced and bacterial endotoxins that can directly invade tissues cause inflammation of the gingiva and periodontium (Wiggs & Lobprise, 1997). The inflammatory response occurs thanks to mediators that recruit and activate cells of the immune system. These defenders assemble and activate other proteins and chemical messengers. Some of these messengers are pyrogens and cause an increase in tissue temperature. Some factors cause local swelling by increasing vascular permeability. Increased vascular permeability allows agents of the immune system to enter damaged tissues (Reiter & Harvey, 2010). This inflammation leads to gingivitis, which initially only damages the gingival tissues. If the inflammation is not treated, this leads to the destruction of periodontal tissue and the structures that support the tooth. Thus, it causes the formation of periodontitis, which is the irreversible stage of the disease (Wiggs & Lobprise, 1997).

The gingival vascular system is a microcirculation zone. Increased vascular density in the gingiva is associated with some of the first non-specific defenses against periodontitis. The capillaries of the FG are the first to react when gingivitis occurs (Nuki & Hock, 1974). Anatomically, the periodontal vascular system is diverse. It is stated that along the length of the periodontium, it also differs along the mesial, distal, oral and buccal aspects of the alveoli (Mörmann et al., 1985). Differences in the position of fine capillaries between incisive and premolar teeth in dogs have also been demonstrated (Söderholm

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& Egeleerg, 1973). In human temperature measurements, it is stated that gingival pockets, different points around the same tooth, temperatures between different tooth types, temperatures between maxillary and mandibular regions differ due to the variation in the functional status of blood flow (Maeda et al., 1979; Mukherjee, 1981). In thermometric studies, it is observed that there is a difference between the rewarming times of the gums of patients with healthy gingiva and periodontitis, which were previously cooled by cold airflow. It is stated that the warming time of the tissues in patients with clinically healthy gingiva is faster than in patients with periodontitis. It has been reported that the slower warming time of the tissues of patients with periodontitis is due to the pathophysiological feature of the relevant vascular system (Mörmann et al., 1985). Acute inflammation, chronic inflammation, and periodontitis in the gingiva in dogs are associated with typical changes in the microvascular system (Hock et al., 1980).

Changes in the vascular circulation in living organisms cause an increase or decrease in tissue temperature, allowing us to evaluate the state of the tissue in which the change is observed (Kunc & Knizkova, 2012). The use of thermography also plays an important role in monitoring the efficacy of treatment in dental-oral diseases and systemic diseases (Mörmann et al., 1985). Temperature is a valuable and objective finding for the diagnosis of periodontal disease, as changes in blood flow also cause changes in local tissue temperature. For this reason, it is thought that the change in tissue temperatures can also eliminate the misconceptions arising from subjective observations (Barnett et al., 1989; Păunică et al., 2009). In addition, the American Academy of Thermology states that infrared thermal imaging, as a rapid diagnostic tool, has a definite benefit in monitoring dental and oral health conditions also emphasizes that it would be appropriate to include this technique in clinical medicine (Schwartz et al., 2015).

Anatomical and histological changes occur in the gingival microcirculation during the initial and formation stages of gingivitis (Kunc & Knizkova, 2012). The increase in local blood flow as a result of the vascular reaction causes an increase in gingival temperature (Maeda et al., 1979). When interpreting the thermographic images of the oral cavity, it is accepted that a pathological condition is diagnosed when the temperature difference exceeds 0.5°C (Dobrzyński et al., 2014). It was also reported that it facilitates the determination of the degree of inflammation of the gingival tissues in dogs (Yiğitarslan et al., 2022). It is reported that the resting dental papilla in healthy gingiva has a higher blood flow than the FG. Gingival blood flow was observed to be significantly lower in patients with chronic periodontitis compared to healthy individuals (Nakamoto et al., 2012). It is suggested that this is due to the decrease in vascularization in chronic diseases. It has been reported that from early periodontitis, thermal properties, including the acute phase, have a higher temperature associated with inflammation (Haffajee et al., 1992). The mean gingival pocket temperature is 33.9±0.4ºC in people who appear clinically healthy; The mandibular gingiva was found to be 0.7±0.2ºC higher than the maxillary gingiva. It is also stated that molar teeth have a temperature profile 1.5±0.3ºC higher than incisive teeth (Ng et al., 1978). It is stated that FG has a lower temperature than AG and AM in dogs with healthy gingiva (Yiğitarslan et al., 2022).

In this study, it was aimed to determine the reference ranges of FG, AG and AM temperatures of dogs with healthy gingiva with a thermal camera. In addition, it was aimed to determine the change in gingival temperatures according to different clinical findings.

MATERIAL and METHODS

Animal Material

In this study, 140 non-brachiocephalic dogs with healthy gums, aged 2-11 years (81 females, 59 males), 86 crossbreds and 54 different breeds were used.

Tools Used in Gum Examination

Periodontal probe was used to determine the gingival bleeding of the dogs and to measure the gingival pocket depths. Thermal camera (Trotec® EC060V, France) was used to take gingival thermograms.

Equipment Used in Anesthesia

An anesthesia device with automatic ventilator and double vaporizer (Draeger Primus®, Draegerwerk AG&Co. KGaA, Germany) was used for general anesthesia of dogs. 0.1 mg/kg diazepam (Diazem® IM/IV, 10 mg/2 ml, Deva, Istanbul) was administered as preanesthetic and 3 mg/kg propofol (Propofol® 1%, Fresenius, Germany) was administered intravenously for induction. A disposable endotracheal tube (Rüsch®, Willy-Rüsch, Germany) was used to ensure the patency of the respiratory tract. Sevoflurane (Sevoflurane®, USP, United States) was used as an inhalation anesthetic for maintenance of anesthesia.

Taking Thermograms

Under the general anaesthesia, dogs were placed in the lateral position. Thermograms were taken with a thermal camera to measure the temperature of the buccal gingival surface. First, the lips covering the tooth surface were removed and the teeth and gums were made visible. Then it was waited for 30 seconds for the temperature to stabilize. After the first image was taken, thermographic images of the buccal gingiva of the right maxillary and mandibular half were taken from a distance of 20 cm for 120 seconds at 30-second intervals. The same procedure was repeated for the left maxillary and mandibular half.

Recording of Clinical Examination Findings

According to the GI in Table 1, clinical findings such as redness, edema and bleeding in the gums were evaluated and recorded (Löe & Silness, 1963). The degree of disease was determined according to the index system. Dogs with tooth mobility and gingival furcation were not included in the study. After the clinical findings of the gums of the dogs determined to have healthy gums were recorded in the examination form, the dogs were discharged.

Table 1. Gingival indeks (Löe & Silness,1963)

Grade	Clinical Finding
0	Healthy gums, no inflammation.
1	Mild inflammation; slight change in color and slight edema. No bleeding on probing.
2	Moderate inflammation. There is edema, redness, shine and bleeding on probing.
3	Severe inflammation. There is edema, redness and ulceration are present. There is spontaneous bleeding.

Evaluation of Thermograms

Thermal images obtained with thermography camera were analyzed with the IC IR Report Software® program. The temperature values of the FG, AG and AM at each tooth level were determined linearly and recorded.

Statistics

IBM SPSS 20 program was used in the analysis of the data. In the evaluation of normal distribution, skewness and kurtosis values were considered. As a result of the analysis, the data with skewness and kurtosis values between -1.5 and +1.5 were normally distributed. ANOVA Tukey test was used to control the importance of the temperature difference between the index grades of the gingival regions. $p<0.05$ was considered statistically significant.

RESULTS

The study included 140 dogs, 86 crossbreds and 54 mixed breeds. 81 of the cases were female and 59 of them were male dogs. It was determined that their body weight was between 15-72.3 kg (26.01±9.58) and their age was between 2-11 years (3.45±1.54).

In the maxilla, 1708 teeth and their gums were examined. 427 of them were grade 0 GI (no bleeding, hyperemia and edema), 432 grade 1 GI (no bleeding, hyperemia and edema are present) and 849 grade 2 GI (hemorrhage). Since spontaneous bleeding was not observed in any of the gingiva, there is no grade 3 GI. Average temperature values of different gingival regions are shown in Table 2**.** The results of the analysis are given in Table 3**.** The temperature difference (r) between the gums of dogs with grade 0 and grade 1 GI was not found to be significant. Statistically significant temperature difference was observed between grade 0 GI and grade 2 GI in FG (r= 0.35°C and $p<0.05$), AG (r= 0.33°C and $p<0.05$) and AM (r=0.29°C and $p<0.05$).

In the mandible, 1893 teeth and the gums of these teeth were examined. 671 of them were evaluated as grade 0 GI, 463 as grade 1 GI and 759 as grade 2 GI. Since spontaneous bleeding was not observed in any of the gingiva, there is no grade 3 gingiva. Average temperature values of different gingival regions are shown in Table 4. The results of the analysis are given in Table 5. Grade 0 GI gingival temperature was statistically significantly different from grade 1 GI and grade 2 GI gingival temperatures ($p<0.05$). However, no significant difference was observed between grade 1 GI and grade 2 GI gingival temperatures ($p>0.05$). There is a positive correlation between mandibular FG ($p<0.001$), AG ($p<0.001$) and AM ($p< .000$) temperature values and GI grades. As the index grade increased, a significant increase was observed in gingival temperatures ($p<0.05$).

Temperature difference between maxillary and mandibular FG (r= 0.78°C and p= .000), AG (r= 0.52°C and p= .000) and AM (r= 0.51° C and p= .000) in dogs with grade 0 GI were found to be statistically significant. Temperature difference between maxillary and mandibular FG (r= 0.58°C and p= .000), AG (r= 0.38°C and p= .000) and AM (r= 0.29°C p= .000) were statistically significant in dogs with grade 1 GI. Temperature difference between maxillary and mandibular FG (r= 0.32°C and p= .000) and AG (r= 0.12°C and p= 0.024) in dogs with grade 2 GI were statistically significant. The temperature difference between maxillary and mandibular AM temperatures ($p>0.05$) were not statistically significant. Maxillary gingival temperature was higher than mandibular gingival temperature in all groups with significant difference.

The temperature difference of FG, AG and AM, which are different anatomical regions of the gingiva, was determined. The temperature difference between different index grades of gingivitis were also determined (Figure 1).

Table 2. Surface temperatures of the maxillary gingiva according to gingival index

Indeks Grade	n	FG Temperature (°C)	AG Temperature (°C)	AM Temperature (°C)
0	427	$36.25±1.43^{A,a}$	$36.81±1.37^{A,b}$	$37.53±1.30^{A,c}$
1	432	$36.40±1.32^{A,a}$	$36.95±1.21^{A,b}$	$37.57±1.21^{A,c}$
2	849	$36.05±1.16^{B,a}$	$36.62±1.08^{B,b}$	$37.28±1.07^{B,c}$
3	0	-	-	-

There is a statistical difference between different uppercase superscripts in the same column ($p<0,05$).
There is a statistical difference between different lowercase superscripts on the same line ($p<0,05$).
n: Number of cases.

Tablo 3. Statistical analysis of gingival temperatures according to gingival index grade in the maxilla

Dependent Variables	(I) Indeks Grade	(J) Indeks Grade	Mean Temperature Difference (I-J)	Standard Error	p
Free Gingiva	0	1	-.15117	.08733	.194
		2	.20331*	.07593	.020
	1	0	.15117	.08733	.194
		2	.35448*	.07563	.000
	2	0	-.20331*	.07593	.020
		1	-.35448*	.07563	.000
Attached Gingiva	0	1	-.14340	.08163	.185
		2	.18474*	.07097	.025
	1	0	.14340	.08163	.185
		2	.32814*	.07069	.000
	2	0	-.18474*	.07097	.025
		1	-.32814*	.07069	.000
Alveolar Mukosa	0	1	-.03571	.08009	.896
		2	.24927*	.06963	.001
	1	0	.03571	.08009	.896
		2	.28498*	.06936	.000
	2	0	-.24927*	.06963	.001
		1	-.28498*	.06936	.000

ANOVA Tukey Test (* p<0.05)

Tablo 4. Surface temperatures of the mandibular gingiva according to gingival index

Indeks Grade	n	FG Temperature (°C)	AG Temperature (°C)	AM Temperature (°C)
0	671	$35.46\pm1.42^{A,a}$	$36.26\pm1.35^{A,b}$	$37.01\pm1.22^{A,c}$
1	463	$35.82\pm1.26^{B,a}$	$36.57\pm1.16^{B,b}$	$37.27\pm1.10^{B,c}$
2	759	$35.73\pm1.21^{B,a}$	$36.50\pm1.12^{B,b}$	$37.28\pm1.00^{B,c}$
3	0	-	-	-

There is a statistical difference between different uppercase superscripts in the same column (p<0,05).
There is a statistical difference between different lowercase superscripts on the same line (p<0,05).
n: Number of cases.

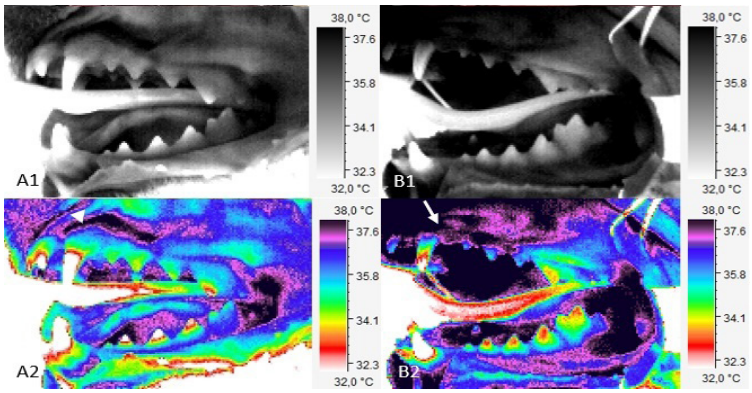

Figure 1. Color distribution of the thermographic image of healthy gingiva (A1, A2) and gingivitis (B1, B2) in dogs (Yiğitarslan et al. 2022)

Tablo 5. Statistical analysis of gingival temperatures according to gingival index grade in the mandibula

Dependent Variables	(I) Indeks Grade	(J) Indeks Grade	Mean Temperature Difference (I-J)	Standard Error	p
Free Gingiva	0	1	-.34542*	.07846	.000
		2	-.25689*	.06885	.001
	1	0	.34542*	.07846	.000
		2	.08854	.07647	.479
	2	0	.25689*	.06885	.001
		1	-.08854	.07647	.479
Attached Gingiva	0	1	-.28751*	.07313	.000
		2	-.21812*	.06417	.002
	1	0	.28751*	.07313	.000
		2	.06939	.07127	.594
	2	0	.21812*	.06417	.002
		1	-.06939	.07127	.594
Alveolar Mukosa	0	1	-.26200*	.06715	.000
		2	-.26468*	.05892	.000
	1	0	.26200*	.06715	.000
		2	-.00268	.06544	.999
	2	0	.26468*	.05892	.000
		1	.00268	.06544	.999

ANOVA Tukey Test (* $p<0.05$)

DISCUSSION

Edema, hyperemia and bleeding in the gingival margin are the most important findings of gingivitis (Gorrel, 2013). There is no recession, furcation or tooth mobility in the gingiva (Gorrel, 2013). Animals with gingival furcation and gingival mobility were not included in this study.

If the progression of gingivitis is not prevented, infection in the periapical region of the tooth root leads to pulpitis and tooth loss. Endodontic diseases arise as a result of severe periodontal diseases (Reiter & Harvey, 2010). However, early diagnosis and treatment is crucial because this situation causes many important health problems in the animal before tooth loss (Niemiec, 2013). Grade 0 refers to healthy gums. Grade 1 represents the gingiva with edema and hyperemia. However, a specialist physician can distinguish at first view the difference between healthy gingiva and hyperemic and edematous gingiva Since bleeding is an easily distinguishable clinical finding, it will be easier to identify grade 2 gingiva. In this study, buccal gingival surface temperature of grade 0 and grade 1 GI dogs was compared. Thus, the effectiveness of the thermal camera was evaluated in the diagnosis of dogs with gingival inflammation but no bleeding.

The gingival vascular system is a microcirculation zone. Increased vascular density in the gingiva is associated with some of the first non-specific defenses against periodontitis. The capillaries of the FG are the first to react when gingivitis occurs (Nuki & Hock, 1974). Anatomically, the periodontal vascular system is diverse. It is stated that along the length of the periodontium, it also differs along the mesial, distal, oral and buccal aspects of the alveoli (Mörmann et al., 1985). In this study, the variation of buccal gingival surface temperatures according to the index grade was determined. Temperature difference between grade 0 and grade 2 in the maxillary FG ($r= 0.35°C$ and $p<0.05$), AG ($r= 0.33°C$ and $p<0.05$), and AM ($r = 0.29°C$ and $p<0.05$) had a significant decrease was observed. In the mandible, the grade 0 GI gingival temperature was statistically significantly different from the grade 1 and grade 2 gingival temperatures ($p<0.05$). There is a positive correlation between mandibular FG ($p<0.001$), AG ($p<0.001$) and AM ($p<.000$) temperature values and GI grade. In other words, as the grade of gingival index increased in the mandible, a significant increase was observed in gingival temperatures ($p<0.05$). In addition, in this study, the temperature difference of FG, AG and AM, which are different anatomical regions of the gingiva, was determined. The temperature difference between different grades of gingivitis was also determined. This was thought to be due to the different anatomic regions having different vascular densities. It was thought that vascular density changed according to the degree of disease.

When interpreting the thermographic images of the oral cavity, it is accepted that a pathological condition is diagnosed

when the temperature difference exceeds 0.5°C (Dobrzyński et al., 2014). However, in the maxilla, FG (r= 0.35°C and p<0.05), AG (r= 0.33°C and p<0.05) and AM (r= 0.29°C and p<0,05) temperature difference was found to be an indicator of gingivitis. In the mandible, FG (r= 0.36°C and p<0.05), AG (r= 0.31°C and p<0.05) and AM (r= 0.26°C and p<0,05) temperature difference was found to be an indicator of gingivitis.

Gingival blood flow was observed to be significantly lower in patients with chronic periodontitis compared to healthy individuals (Nakamoto et al., 2012). It is suggested that this is due to the decrease in vascularization in chronic diseases. In a study on gingival surface temperature, it is reported that thermal properties, including the acute phase, have a higher temperature associated with inflammation from early periodontitis (Haffajee et al., 1992). In this study, a significant decrease in maxillary gums was observed in dogs with gingivitis. A significant temperature increase was detected in the mandibular gingiva. It was thought that this situation was caused by the different vascular behaviors in different anatomical regions.

It is stated that the average gingival pocket temperature in people with healthy gums is 33.9±0.4ºC. The mandibular gingiva was found to be 0.7±0.2ºC higher than the maxillary gingiva (Ng et al., 1978). The FG temperature of the buccal surface of the dogs with healthy gingiva in the maxilla was 36.25±1.43°C. The AG temperature was 36.81±1.37°C and the AM temperature was 37.53±1.30°C. In the mandible, in dogs with healthy gums, the FG temperature was 35.46±1.42°C. The AG temperature was 36.26±1.35°C and 37.01±1.22°C. In contrast to the study in humans, the maxillary gingival temperature was found to be higher than the mandibular gingival temperature in dogs with healthy gums. This temperature difference was measured as 0.78°C in FG, 0.52°C in AG and 0.51°C in AM. This situation supports the literature knowledge that different anatomical regions have different vascular densities.

CONCLUSION

In this study, buccal gingival temperatures of dogs with healthy gingiva were determined. It can be stated that different anatomical regions of the gingiva have different vascular density. In addition, temperature changes in the gingiva according to the disease grade were also revealed. Thus, it was analyzed what kind of temperature behaviors could be in order to diagnose gingivitis at an early stage. The sensitive sensors of the thermal camera can detect even the smallest temperature changes. Thus, it provides an advantage to the physician in order to determine possible disease states and to initiate treatment intervention early. As a result, it was concluded that the thermography is an auxiliary diagnostic tool for detecting inflammatory changes in the gingiva in dogs and thus, irreversible diseases such as periodontitis can be prevented.

DECLARATIONS

Ethics Approval

This research was carried out on the basis of the permission of Mehmet Akif Ersoy University Local Animal Ethics Committee dated 13.03.2019 and numbered 504.

Conflict of Interest

The authors declare that there have no conflict of interests.

Consent for Publication

Does not need a publication consent.

Author Contributions

Idea, concept and design: KY, CÖ

Data collection and analysis: CÖ, KY

Draft of the article: CÖ, KY

Critical review: KY, CÖ

Data Availability

The data collected within the scope of the study has not been shared.

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Research Article

Effect of use of potato chips waste as a source of easily soluble carbohydrates in alfalfa silage on silage quality

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ABSTRACT

The goal of this study was to examine the effect of adding potato chips waste, which is a by-product of the potato processing industry, on silage quality, as an easily soluble carbohydrate source, to the alfalfa (Medicago sativa L.) plant, which has a high buffering capacity and is therefore difficult to ensilage. In the study, alfalfa (Medicago sativa) harvested at the 10% flowering point was used as the plant material. Trial groups of silages prepared by adding potato chips waste to alfalfa at the rates of 0% (control), 0.5%, 1% and 2% by wet weight were formed. After the 60-day fermentation period was complete, the silages were opened, and the nutritional compositions and fermentation products were identified. When silages made with various amounts of chips were analyzed, variations between the groups in terms of dry matter (DM), crude ash (CA), acid detergent finer (ADF), and neutral detergent fiber (NDF) content of the silages that were generated by the research were statistically significant. The differences between the silage groups were discovered to be statistically significant, despite the fact that they were not significant in terms of CP content. In the study, reductions were seen as a result of the addition of chips in comparison to the control group when the pH, ammonia nitrogen (NH_3-N), carbon dioxide (CO_2) production, and total yeast mold values of the fermentation properties of the silages were studied. With the addition of 1% chips, an increase in the silages' total lactic acid bacteria (LAB) levels was seen. As a result, when all the parameters were examined, it was determined that the silages prepared by adding 1% chips waste had positive effects on silage fermentation.

INTRODUCTION

Alfalfa (*Medicago sativa*), which is called the queen of forage plants, is oftenly dried and fed to the animals. However, significant nutrient losses occur during drying and storage (McDowell 1989; Oktay et al., 1990). In recent years, besides its use as a dry grass, its silage has also gained importance (Çerçi et al., 1996). Due to the high level of alfalfa protein, it is classified as difficult ensiled feeds (Ergün et al., 1999). It is challenging to obtain quality silages from such feeds. For this reason, different additives are used to ensure the fermentation of silage fodder plants that are low in carbohydrates and rich in protein, but additives are mostly used to account for the carbohydrate deficit in the the subject plant (Güler, 2001; Şahin et al., 1999). The potato (*Solanum tuberosum L.)* plant is a member of the Solanacae family. Potato-producing countries include China, India, Russia, Ukraine and the USA. It was first cultivated in Turkey at the end of the nineteenth century. On the basis of regions in Turkey, it was first cultivated in the Eastern Black Sea region and then in the Western Thrace region (Berksan, 2002). Approximately half of the potatoes produced in the world are consumed fresh, while the other half is used as processed food product, animal feed, industrial starch and seed. Potatoes are mainly processed into frozen products and chips. The remaining amount is consumed in areas such as processed potatoes, animal feed, seed, industrial starch, which have areas of use such as chips and French fries.

Potato skins and the other parts of the potato that remain as waste after consumption contain a high percentage of starch (Özdemir & Malayoğlu, 2017). The potato plant is one of the basic foods consumed for nutrition by the world population and it contains plenty of carbohydrates, proteins, vitamins and minerals in its structure. Therefore, it is a plant with a high nutritional value (Onaran, 2002). As a result of industrial and agricultural applications, large amounts of potato by-products are produced every year. Although the potential properties of these by-products cannot be exploited, these by-products are often left to the environment by businesses who are faced with serious environmental problems (Hofvendahl &Hahn-Hagerdal, 2000). This study aimed to use potato chips waste, which is one of the by-products of the potato processing industry that causes environmental pollution, as an easily soluble carbohydrate source in alfalfa silage.

MATERIAL and METHODS

In the study, alfalfa (*Medicago sativa*) harvested at the 10% flowering point was used as the plant material. The potato chips waste was added to the alfalfa at the rates of 0.5%, 1% and 2% on a wet weight basis. After the alfafa was harvested, it was left to wither up to the DM content of 30 or 25% approx, and it was then chopped in 1.5-2.0 cm dimensions. In the study, four different silage groups were identified and four replications were formed in each group. After adding the potato chips waste to approximately 1 kg of crushed alfalfa samp-

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les, they were placed in 1.5 liter airtight glass jars, their mouths were tightly closed, and they were left for fermentation for 60 days by being ensiled at room temperature. At the conclusion of the 60-day fermentation period, the silages were opened, and the 3-5 cm portions at the jar tops were removed and discarded. After the silages were opened, 25 g of silage sample was crushed in a blender with 100 ml of distilled water and its pH was measured (Polan et al., 1998). Then the obtained filtrates were placed in centrifuge tubes. For ammonia nitrogen analysis, 0.1 ml of 1M HCl was added to the centrifuge tubes and stored at -18°C. Using the method described by Broderick & Kang (1980), ammonia nitrogen analysis of the silage samples were carried out.

Dry matter (DM), crude protein (CP) and crude ash (CA) analyzes were performed according to the Weende analysis system (AOAC, 1990), and ADF and NDF analyzes were performed according to the method reported by Van Soest et al. (1990). The silages were subjected to an aerobic stability test [determination of carbon dioxide (CO_2) production values] for five days (Ashbell et al., 1991). The silage materials and the obtained silages were dried by air drying method and ground in a laboratory mill to pass through a 1 mm sieve, and nutrient composition analyzes were made. The total lactic acid bacteria (LAB) count in the silage material was determined using the method reported by Güney & Ertürk (2020) according to the tempo automatic bacteria counter test method. The total

Table 1. Nutrient analysis of chips waste and siloed alfalfa

Nutrients	DM	CA	CP	ADF	NDF
Alfalfa	31.60	10.65	14.95	25.57	41.04
Chips Waste	88.90	2.88	7.50	3.50	9.86

DM: Dry matter, %; CA: Crude ash, DM%; CP: Crude protein, DM%; ADF: Acid detergent fiber, DM%; NDF: neutral detergent fiber, DM%.

Table 2. Nutrient content of silages prepared by adding chips waste at different rates to alfalfa plant

Groups	DM	CA	CP	ADF	NDF
Control	34.38	11.35	16.30^{a}	23.46	40.33
0.5% Chips Waste	34.32	11.44	16.20^{b}	21.18	41.00
1% Chips Waste	34.54	11.15	16.14^{bc}	21.47	40.59
2% Chips Waste	34.20	11.08	16.08^{c}	22.39	39.12
SEM	0.133	0.095	0.024	0.409	0.320
P	0.870	0.560	0.001	0.195	0.188

a-d:Values with different letters in the same column were found to be different (P<0.05), SEM:Standart Error of Mean; DM: Dry matter, %; CA: Crude ash, DM%, CP: Crude protein, DM%, ADF: Acid detergent fiber, DM%; NDF: neutral detergent fiber, DM%.

Table 3. Fermentation contents of silages prepared by adding chips waste at different rates to alfalfa plant

Groups	PH	NH_3-N	CO_2	Total Yeast-Mold cfu/g	LAB kob/g
Control	5.02^{a}	9.580^{a}	2.755^{a}	8.54^{a}	7.21^{b}
0.5% Chips Waste	4.78^{b}	4.797^{b}	2.012^{b}	6.92^{b}	7.55^{b}
1% Chips Waste	4.56^{c}	3.687^{b}	1.908^{b}	6.34^{c}	7.58^{a}
2% Chips Waste	4.32^{d}	3.750^{b}	1.500^{b}	6.25^{c}	5.89^{b}
SEM	0.069	0.652	0.153	0.240	0.228
P	0.000	0.000	0.012	0.000	0.008

a-d;Values with different letters in the same column were found to be different (P<0.05), SEM:Standart Error of Mean; CO_2: Carbon dioxide formation, g/kg DM, NH_3-N/TN: Ammonia nitrogen, LAB: Lactic Acid Bacteria cfu/g.

amount of yeast and mold contained in the silages were determined using the method reported by Filya et al. (2000).

The data obtained at the end of the research were evaluated with one-way analysis of variance (One-way Anova) using the SPSS statistical software program (SPSS, 2008). Duncan's multiple range test was used to compare the mean between groups. The significance level of differences between groups was made according to $p<0.05$.

RESULTS

The study's chemical composition of alfalfa and potato chips waste used as silage material is presented in Table 1.

The silage nutrient content of the addition of the potato chips waste at different rates (0.5%, 1%, and 2%) to the alfalfa silage is presented in Table 2 and the fermentation characteristics are presented in Table 3.

The differences between the groups were determined to be statistically insignificant ($p>0.05$) when the silages' DM, CA, ADF, and NDF values were investigated in Table 2, but they were statistically significant ($p<0.05$) when it came to CP va-lues.

When the pH, NH_3-N, CO_2, yeast mold and LAB values of the silages were examined in Table 3, the differences between the groups were found to be statistically significant ($p<0.05$). Although the control group's silages had the highest pH value, a drop in pH was seen as a result of the rise in chips waste.

DISCUSSION

In this study, a decrease in the CP values was observed in all the experimental groups due to the increase in chips waste. It was concluded that this downward trend was due to the low protein content of the chips waste added to the alfalfa silage. The reason why there was no significant difference in the ADF and NDF values between the control and experimental groups in this study was thought to be related to the fact that the chips waste could not increase the low LAB activity in the environment and therefore the cell wall components in the silages were not broken down.

Due to the low concentration of water-soluble carbohydrates in the alfalfa plant, the pH values in the additives groups were found to be lower than those in the control group. The pH values of the silages are affected by many factors such as the type of LAB used, the water soluble carbohydrate content of the plant, the dry matter level, and the buffering capacity (Basmacıoğlu & Ergül, 2002). Silo fermentation affects the nutritional value and hygienic structure of silages. The pH formed during fermentation is extremely important and is an important parameter used to determine silage fermentation and silage quality (Filya, 2000).

In the study, when the silage NH_3-N values were compared with the control group, a decrease was observed in the silages prepared with the addition of potato chips waste ($p<0.05$). It is thought that easily soluble carbohydrate sources have a positive effect on silage fermentation and reduce proteolysis (Bingöl, 2009). The total yeast mold values and CO_2 content of the silages with all additives applied were found to be lower than those of the control group ($p<0.05$). The main microorganisms responsible for aerobic deterioration are yeast and molds. Filya (2001) reported that the intense CO_2 production in silages is an indicator of aerobic deterioration of the silages. Filya (2002) also noted that the presence of unused sugars after fermentation reduces the aerobic stability of silages during the period when the silages are opened for use in feeding and are exposed to a completely unlimited air intake. Similarly, Canpolat et al. (2010), reported that the addition of grape pomace as an easily soluble carbohydrate source to alfalfa silage improved the aerobic stability value. When the silages' total LAB values were looked at, an increase was observed with the addition of 1% chips waste, the fact that the highest LAB value (7.58) and the lowest yeast mold value (6.34) were in the silage group with a 1% chips waste addition, the amount of acetic acid produced by the LAB fermentation inhibited the growth of yeast and molds (Driehuis & Elferink, 2000). Canpolat et al. (2013) reported that the addition of gladia fruit as an easily soluble carbohydrate source to alfalfa silage increased the LAB value and reduced the mold, which supports the findings of this study.

CONCLUSION

When all the parameters of the study were examined, it was observed that the silages prepared by adding 1% chips waste had positive effects on silage fermentation. Additionally, it was determined that chips waste can be added to the as an alternative, which can be used to increase the quality of the alfalfa silage, which is difficult to ensilage, and to increase the level of easily soluble carbohydrates in the silage.

DECLARATIONS

Ethics Approval

Not applicable.

Conflict of Interest

The authors declare that they have no competitive interests.

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: BDD

Data collection and analysis: AD, NK, MK, MEA

Drafting of the manuscript: BDD

Critical review: MA

Data Availability

Not applicable

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Research Article

Investigation of possible heavy metals and antibiotic residues in commercial collagen

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ABSTRACT

The current study investigated whether commercial collagens were within physiologically acceptable limits to ensure their safer use. For this purpose, 10 of the 25 most popular collagen from fish and farm animals (FA) sold on the internet were randomly selected and purchased from a pharmacy. The zinc, lead, cadmium, mercury, and arsenic levels in these commercial products were then analyzed by ICP-OES. Streptomycin, sulfonamide, tetracycline, and chloramphenicol levels in the samples were determined by HPLC. No lead, mercury or arsenic residues were found in any of the tested samples. The mean cadmium levels in fish and FA collagen samples were not significant between the groups (P=0.2548). The lowest cadmium level in fish collagen samples was 0.152 mg/kg and the highest cadmium level was 0.288 mg/kg. Cadmium levels detected in FA collagen samples ranged from 0.183 mg/kg to 2.78 mg/kg. The mean zinc levels in fish and FA collagen were not significant (P=0.2644). The lowest zinc level in fish collagen was 1.368 mg/kg and the highest was 2673 mg/kg. The lowest and highest zinc levels in FA collagen were 1.750 mg/kg and 1528 mg/kg, respectively. According to the current results, no streptomycin, sulfonamide, and tetracycline residues were found in any of the collagen samples evaluated. Chloramphenicol was only in two fish collagen samples, but these values were below the lower detection limits. The results indicated that there is no or very low risk of heavy metal and antibiotic residues in commercial collagens sold in our country.

INTRODUCTION

The word collagen originates from Greek: "cola" means chewing gum and "gene" means to produce. Collagen is one of the most produced proteins in humans and many other living organisms. This special molecule is the main structural fibrillar protein found in connective tissues of the skin, tendons, joints, and bones. Thanks to its fibrillar properties, it is responsible for the stability and strength of body tissues by forming support networks between cellular structures (Nimni, 1980). Collagen fibers may be damaged due to aging and may lead to loss of function in many tissues where collagen support is provided. Therefore, it has been extensively investigated as a polymer for use in many biomedical products such as cosmetics and pharmaceuticals (Meena et al., 1999; Sionkowska et al., 2020). The cosmetic industry is making great efforts to incorporate this biomolecule into many existing products for use against skin aging, and there is a great demand for collagen in the food industry, as it has a high protein content and good functional properties such as water absorption capacity and emulsifying ability (Lafarga and Hayes, 2014; Schmidt et al., 2016).

The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. In science, heavy metals are metallic elements that are toxic and have a high density, specific gravity, or atomic weight. The criteria used and whether to include metalloids vary by author and context. In metallurgy, for example, a heavy metal may be defined on the basis of density, in physics, the distinguishing criterion may be the atomic number, while a chemist would probably be more interested in chemical behavior. Nowadays, the term is commonly used in a slightly different sense, referring to any metal that can cause health problems or environmental damage. A commonly used criterion for heavy metals is a density greater than 5 g/cm^3. Examples of heavy metals include mercury, cadmium, arsenic, zinc, and lead (Pourret et al., 2021).

Antibiotics have been used since time immemorial. There is also good historical evidence that ancient civilizations used a variety of naturally available treatments for infections, such as herbs, honey, and even animal feces (Keyes et al., 2003). The term "antibiosis", meaning "against life", was coined by the French bacteriologist Jean Paul Vuillemin as a descriptive name for the phenomenon exhibited by these first antibacterial drugs (Foster and Raoult, 1974). Antibiosis was first described in bacteria in 1877 when Louis Pasteur and Robert Koch observed that an airborne bacillus inhibited the growth of Bacillus anthracis (Saxena, 2015). Since then, the efficacy and easy access to antibiotics has led to their overuse (Laxminarayan et al., 2013) and some bacteria have developed resistance to them (Gualerzi et al., 2013). The World Health Organization has classified antimicrobial resistance as a widespread serious threat. Global deaths attributable to antimicrobial resistance totaled 1.27 million in 2019 (Murray et al., 2022).

Mainly derived from farm animals and seafood, the use of

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collagen today has become attractive for both cosmetic and medical use. However, global pollution poses a risk to collagen sources. Therefore, while consuming collagen for a more conscious diet and a better life, it is possible to unknowingly be exposed to heavy metal and/or antibiotic residues. Research on heavy metal and antibiotic residues in commercially available collagen products have only recently begun. Studies on the safety of collagen products in terms of heavy metal and antibiotic residues are insufficient and the safe production process has yet to be completed. Therefore, the current study aimed to investigate some heavy metal and antibiotic residues that are likely to be found in commercial collagens on sale in Turkey.

Table 1. Limit of detection (LOD), wavelength and R2 values used in ICP-OES analysis.

	Heavy Metals				
	Zn	Pb	Cd	Hg	As
LOD (µg/L)	5	5	5	1	5
Wavelength (nm)	213.85	220.353	226.502	253.652	188.9
R^2	0.999	0.999	0.999	0.999	0.999

Zn=Zinc, Pb=Lead, Cd=Cadmium, Hg=Mercury, As=Arsenic.

MATERIAL and METHODS

The current study was supported by Mehmet Akif Ersoy University, Scientific Research Projects Coordination Office with project number 0767-YL-21.

Collection and Storage of Samples

A total of 4 most popular online shopping site in Turkiye was chosen according to the 2021 values. From these online sites, one was randomly selected by drawing method. On this online site, collagen brands from farm animals (FA) and fish without mineral additives were listed separately using a popularity filter. Within this list, 10 of the fish and FA collagen brands in the top 25 were selected randomly by drawing method. These brands were purchased from a local pharmacy in the Burdur city center. The collagens were kept at -5 °C until the analysis was performed.

Analysis of Heavy Metal and Antibiotic Levels in Samples

Heavy metal and antibiotic analyses of the samples were carried out in the laboratories of Mehmet Akif Ersoy University, Scientific and Technology Application and Research Center. Zinc, lead, cadmium, mercury, and arsenic levels in the samples were analyzed using an inductively coupled plasma optical emission spectrometer (Perkin Elmer ICP-OES Optima 8000). The limit of detection (LOD), wavelength, and R2 values are given in Table 1.

The chromatogram values of the samples tested for antibiotics are in Table 2. Shimadzu Prominence Brand HPLC instrument was used for antibiotic measurements. Antibiotic standards used for the analyses were purchased from Sigma-Aldrich (St. Louis, MO, USA) as Vetranal™ analytical grade standards (certified purity >95%). Methanol (MeOH), ammonium formate, acetonitrile (ACN), and formic acid (HPLC grade >95%) were purchased from Merck (Darmstadt, Germany). Disodium ethylenediaminetetraacetate (Na2EDTA) was obtained from Sigma Aldrich (St. Louis, MO, USA).

Table 2. Limit of detection (LOD), wavelength and calibration function values used in HPLC analysis.

	Antibiotics			
	Streptomycin	Sulfonamide	Tetracycline	Chloramphenicol
LOD (ppb)	1.21	3.11	1.48	2.13
RT	1.9	4.5	2.6	3.4
Wavelength (nm)	190	190	270	280
Calibration function	$Y=1.11*10^{-6}x+7.43*10^{-5}$	$Y=2.13*10^{-6}x+3.18*10^{-5}$	$Y=3.14*10^{-6}x+2.22*10^{-4}$	$Y=4.33*10^{-6}x+3.12*10^{-5}$

Statistics

T Test procedure of SAS statistical program was used for statistical evaluation where available.

RESULTS

The amounts of heavy metals tested from the samples are shown in Table 3. No lead, mercury or arsenic residues were found in any of the analyzed samples. The lowest cadmium level in fish collagen samples was 0.152 mg/kg and the highest cadmium level was 0.288 mg/kg (mean 0.2495±0.0393). The levels detected in collagen samples of farm animal origin (FA) were also low and ranged from 0.183 mg/kg to 2.78 mg/kg (mean 0.2311±0.0301). The mean cadmium levels in fish and FA collagen samples were not significant between groups (P=0.2548).

The distribution of zinc levels in the samples showed very

Table 3. Heavy metal concentrations in the samples (mg/kg).

Sample	Zn	Pb	Cd	Hg	As
F1	2.118	ÖA	0.152	UDL	UDL
F2	3.424	UDL	0.248	UDL	UDL
F3	1.546	UDL	0.277	UDL	UDL
F4	1.368	UDL	0.261	UDL	UDL
F5	5.831	UDL	0.222	UDL	UDL
F6	1935.0	UDL	0.268	UDL	UDL
F7	910.50	UDL	0.288	UDL	UDL
F8	4.169	UDL	0.276	UDL	UDL
F9	1.973	UDL	0.242	UDL	UDL
F10	2673.0	UDL	0.261	UDL	UDL
FA1	4.480	UDL	0.257	UDL	UDL
FA2	2.204	UDL	0.278	UDL	UDL
FA3	3.133	UDL	0.240	UDL	UDL
FA4	1.770	UDL	0.246	UDL	UDL
FA5	1528.0	UDL	0.214	UDL	UDL
FA6	2.938	UDL	0.190	UDL	UDL
FA7	5.200	UDL	0.237	UDL	UDL
FA8	4.059	UDL	0.183	UDL	UDL
FA9	2.654	UDL	0.215	UDL	UDL
FA10	4.718	UDL	0.251	UDL	UDL

F1-F10=Fish collagen samples, FA1-FA10, Farm animal collagen samples, UDL=Under detection limits, Zn=Zinc; Pb=Lead, Cd=Cadmium, Hg= Mercury, As=Arsenic.

Table 4. Antibiotic residue amounts found in the samples (mg/kg).

Sample	Streptomycin	Sulfonamide	Tetracycline	Chloramphenicol
F1	UDL	UDL	UDL	UDL
F2	UDL	UDL	UDL	UDL
F3	UDL	UDL	UDL	UDL
F4	UDL	UDL	UDL	UDL
F5	UDL	UDL	UDL	UDL
F6	UDL	UDL	UDL	UDL
F7	UDL	UDL	UDL	UDL
F8	UDL	UDL	UDL	UDL
F9	UDL	UDL	UDL	0.001
F10	UDL	UDL	UDL	0.001
FA1	UDL	UDL	UDL	UDL
FA2	UDL	UDL	UDL	UDL
FA3	UDL	UDL	UDL	UDL
FA4	UDL	UDL	UDL	UDL
FA5	UDL	UDL	UDL	UDL
FA6	UDL	UDL	UDL	UDL
FA7	UDL	UDL	UDL	UDL
FA8	UDL	UDL	UDL	UDL
FA9	UDL	UDL	UDL	UDL
FA10	UDL	UDL	UDL	UDL

F1-F10=Fish collagen samples, FA1-FA10, Farm animal collagen samples, UDL=Under detection limits.

different levels of variation among the samples. The lowest zinc level in fish collagens was 1.368 mg/kg and the highest was 2673 mg/kg with a group mean of 553.89 (±498.53). In FA collagen, the lowest and highest zinc levels were 1.750 mg/kg and 1528 mg/kg, respectively, and the group means was 155.92 (±148.49). There is a significant fluctuation in zinc levels between both fish and FA collagen samples. In addition, statistically, the average zinc content in fish and FA collagen was found to be insignificant (P=0.2644).

The results of antibiotic residues measured in fish and FA collagen are in Table 4. According to the results, no streptomycin, sulfonamide, or tetracycline residues were found in any of the collagen samples evaluated. Chloramphenicol was detected in only two fish collagen samples and these values were below the lower detection limit.

DISCUSSION

According to a study from Nutrition Business Journal, the international collagen market was worth approximately $1 billion in 2019 and is estimated to have a yearly growth rate of 7.7%. Worldwide, the market could reach $6.5 billion by 2025 as collagen continues to be incorporated into more foods and beverages, topical products, and even used for patient treatment (Watrous, 2020). The major factors driving the progression of the collagen market include the increasing demand for dietary supplements, the growing adoption of collagen in the food and beverage industry, and the growing trend of consumers toward healthy and protein-rich diets. In essence, the growth of the protein powders and collagen market is further driven by the consumer perspective that food is medicine and medicine is food.

No studies on heavy metal contamination in collagens were found in the current literature. However, Consumer Reports (2010) purchased 15 of the best-selling protein powders in the US and tested them for heavy metals. While the results revealed that levels of heavy metals did not pose a threat to human health, the levels detected in a few products were at alarming levels. Consuming three servings a day of some products was found to be enough to cause daily exposure to arsenic, cadmium, or lead in excess of recommended limits. The study concluded that cadmium is particularly important since it accumulates in different organs and causes damage specifically in the liver and kidneys. Also in 2018, the Clean Label Project, a national non-governmental organization, conducted a study of 134 protein powder products of animal origin and screened these products for heavy metals, bisphenols, and pesticides. The study found that 70% of the best-selling protein powders in the US had lead levels up to 123.5 ppm and 74% had cadmium levels up to 306 ppm (Organic Consumer Reports, 2020).

In a recent study, some heavy metals were also detected in krill oils, one of the popular food supplements today (Kızılırmak et al., 2022). Although cadmium (1.0 mg/kg) and mercury (0.1 mg/kg) levels of the tested samples of 11 randomly selected krill oil brands were found to comply with the standard limits, all of the tested krill oils contained lead above the tolerable lead limits (0.08 mg/kg) specified by Codex Alimentarius for food supplements. Furthermore, only 1 sample had arsenic levels below acceptable limits (0.1 mg/kg). The results showed that krill oils in Turkiye may pose a potential threat to public health in the long term (Kızılırmak et al., 2022).

The same concerns apply to collagen since it is produced from animal sources. In a study conducted by the Clean Label Project in the USA, 30 collagen products were tested for arsenic, cadmium, lead, and mercury. It was reported that the amount of mercury was below the detectable level in 66% of the supplements tested, and although trace amounts of mercury were detected in the remaining 34%, these values were lower than 8ppm and did not pose public health. Moreover, arsenic, cadmium, and lead residues were not detected in 36%, 83%, and 63% of the samples tested. Although 64% of the collagen samples contained measurable concentrations of arsenic ranging from 0.09 to 4.7 µg within the recommended daily allowance, these values were below the State of California level of 10 µg. Cadmium was found in 17% of the collagen products tested, ranging from 0.23 to 9.17 µg in the recommended daily allowance of collagen. This 4.1 µg dose was more than twice the daily limit for the state of California. Lead was measured in 37% of the products tested at levels ranging from 0.09 to 1.57 µg in the recommended daily allowance of collagen. The amount of lead found in the 4 products investigated was 2 to 3 times higher than the maximum acceptable level of 0.5 µg. One of the products tested failed to meet the safety criteria in both the cadmium and lead categories. In addition, four out of a total of 30 products (13%) failed to meet the standards for maximum limits, which the Collagen Stewardship Alliance characterizes as "clearly unacceptable" (Clean Label Project, 2021).

In contrast to these studies, it is pleasing to note that none of the samples in our study had detectable levels of mercury, lead, and arsenic, while cadmium was found at minimal levels. Mercury is a naturally occurring metal in the environment. However, mercury can be found in different parts of the world due to agricultural processes, industrial applications, manufacturing, and pollution (Gworek et al., 2020). Lead is also a toxic heavy metal. It has many industrial applications and was commonly used in paint and water pipes before modern trends were established. Like other toxins, lead can leach into water and soil and contaminate food. Like other toxins, lead can leach into water and soil and contaminate food (Bouchard et al., 2009). Arsenic is a naturally occurring element on the earth's surface. However, it is also used in industrial settings such as smelting and mining operations. Arsenic is very toxic to animals and humans. It can leach into soil and water and can be absorbed by plants. Aquatic animals, red and white meat, dairy products such as milk and yogurt, and grains are the main sources of dietary arsenic. In areas where arsenic is naturally present at high levels, foods such as rice prepared with high-arsenic water and food crops irrigated with contaminated water also contribute to the total daily intake (Grund et al., 2008). As a toxic natural element found worldwide cadmium can be found in our food chain through industrial pollution of water sources and topsoil. Thus, grains, vegetables, fruits meats, seafood, and protein drinks can be contaminated by cadmium (EFSA, 2012). In this context, the absence of these heavy metals in the collagen products tested in our study shows that

these products sold in Turkey do not pose a health threat in terms of heavy metals.

In our study, different levels of zinc were found in collagen samples. Zinc is known as an essential mineral that has been used as a dietary supplement for recent years. It can be found naturally in foods. Zinc is also used in some cold medicines and denture adhesive products. Zinc is an important mineral that has a part in cellular metabolic events. It is essential for the catalytic activity of many enzymes and plays a role in enhancing immune function. Zinc is crucial for growth and development (Ryu and Aydemir, 2020), it is essential for cell division and signaling, DNA and mRNA synthesis, and wound healing (Ryu and Aydemir, 2020; King and Cousins, 2014). Thus, the zinc values obtained from the samples analyzed did not pose a health risk in general. On the other hand, in the collagen products tested, there was no indication on the labels that they contained zinc. Therefore, the relatively high zinc levels in some samples may pose a health problem for people taking additional zinc as a food supplement. High zinc intake can cause headaches, upset stomachs, nausea, loss of appetite, and dizziness (Ryu and Aydemir, 2020; King and Cousins, 2014). Zinc consumption for longer time periods for doses of 50 mg or more (usually from overuse of zinc-containing denture adhesive products or supplements) might lower HDL cholesterol concentration hamper copper absorption and negatively affect immune function (King and Cousins, 2014; Plum et al., 2010; Ryu and Aydemir, 2020). Higher doses of zinc up to 142 mg/day could also impede magnesium absorption and disturb magnesium homeostasis (Spencer et al., 1994). Therefore, unintentional excess zinc intake can be detrimental to an individual's health and should therefore be stated on the label of food supplements such as collagen.

The use of antibiotics is essential in the prevention and treatment of animal diseases (Darwish et al., 2013). Nowadays, antibiotics are used in animals not only to treat diseases but also to improve animal production. Antibiotics improve growth and feed efficiency and reproductive performance and synchronize the reproductive cycles. Growth improvement due to antibiotics was first described in the mid-1940s and growth-promoting antibiotics became common practice in animal practice. Antibiotics are among the most important compounds involved in animal feed production. Approximately 80% of farm animals used in food production are treated with antibiotics during their lifetime (Pavlov et al., 2008). However, The World Health Organization (WHO) has called for a ban on growth-promoting antibiotics, arguing that antibiotic usage could cause a variety of health problems in individuals (Graham et al., 2007). This is because, if not used with caution, these antibiotics can cause residues to accumulate in animal products such as milk, cheese, eggs, and meat that are not allowed in foods intended for human consumption.

Antibiotics can enter the human body in different ways, either directly or indirectly through use in animals as growth stimulants, disease prevention and treatment, and contamination (Phillips et al., 2004). A recent study ranked antibiotic use in farm animals by country. China and United States ranked first and second places with 23% and 13%, respectively. The ranking of antibiotic use in farm animals by other countries was 9% for Brazil, 3% for India, and 3% for Germany (Van Boeckel et al., 2015). Antibiotic residues are metabolites present in trace amounts in any edible part of animal products after antibiotic administration. Antibiotic residues greater than the maximum tolerable limit in food animals may contribute to the development of antibiotic resistance in animals or humans. In this context, the absence of antibiotic residues in commercially available collagen is important for public health. In our literature review, no study investigating possible antibiotic residues in collagen products was found. The data obtained from our study revealed that sulfonamide, chloramphenicol, tetracycline, and streptomycin were not found in either fish or bovine collagen supplements.

CONCLUSION

The results of this study showed that commercial collagens (from livestock and fish) selected by random sampling in Turkiye do not contain heavy metals (zinc, lead, cadmium, mercury, and arsenic), and antibiotics (streptomycin, sulfonamide, tetracycline, and chloramphenicol) residues tested at a level that may threaten public health. Our study also revealed that zinc, a useful metal for mammals, were present in some samples at considerable levels. Although not at toxic levels, these levels of a metal that is not listed on the label may cause health problems in people who take zinc as a food supplement.

DECLARATIONS

Ethics Approval

Not applicable.

Conflict of Interest

The authors declare that they have no conflict of interests.

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: DD, OYG

Data collection and analysis: DD, OYG

Drafting of the manuscript: DD, OYG

Critical review: DD, OYG

Data Availability

Data supporting the findings of this study are available fromthe corresponding author upon reasonable request.

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Research Article

A morphometric comparison of the skulls of Akkaraman and Kangal Akkaraman sheep on a three-dimensional model using computed tomography

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ABSTRACT

This study was conducted to determine the craniometric characteristics of the skulls of Akkaraman and Kangal Akkaraman sheep, local breeds in Turkey, using computed tomography (CT). The study material comprised two groups of 12 heads of healthy male Akkaraman and Kangal Akkaraman sheep, aged 8-12 months. The heads were scanned with a CT device, then these images were converted into a three-dimensional structure using the 3D Slicer program and morphometric measurements were calculated. A total of 13 parameters and 5 indexes were measured in each skull, and the morphometric differences between the skulls of Akkaraman and Kangal Akkaraman sheep were determined using statistical methods. All the characteristics examined were expressed as mean±SD values. The study results of the craniometric data showed a statistically significant difference in the parameters of skull length, skull width, greatest length of the nasal bone, greatest breadth across the nasal, medial frontal length, cranial width, facial width, height of the foramen magnum, greatest breadth of the foramen magnum, greatest frontal breadth and least breadth between the orbits ($p<0.05$). No statistically significant difference was observed between the breeds in terms of viscerocranium length, greatest inner width of the orbit, and craniofacial indexes ($p>0.05$). These data can be considered useful for veterinarians in the fields of surgery and clinical practice, and for studies in the field of zooarchaeology, and sheep taxonomy.

INTRODUCTION

Sheep farming, which is an important economic area, is widespread in many parts of the world in areas with vast meadows and pastures and dry climatic conditions. Sheep breeding has become the most profitable branch of livestock production due to the structure and ability of sheep to make the best use of areas with large pastures of low quality. Sheep breeding has an important place in Turkey due to the soil structure and the suitability of grazing opportunities. The Akkaraman sheep breed is intensively bred in Turkey, especially in Central Anatolia, and accounts for almost half the current sheep population (Akçapınar, 2000). The Kangal Akkaraman sheep breed, which was previously recognised as a variety within the Akkaraman sheep breed, one of the indigenous breeds of Turkey, was registered as an independent breed with the communiqué number 28384 published in the Official Gazette on August 14, 2012 (Oğrak et al., 2014). Compared to the Akkaraman breed and other breeds, the Kangal Akkaraman sheep has a larger structure and higher productivity and is compatible and resistant to the natural conditions of the region. Moreover, it is a combined breed for both meat and milk production. It is distinguished from the Akkaraman sheep breed and other varieties by black spots around the eyes, on the feet and around the shins, and by the convex nasal area (Örkiz et al., 1984). Skull morphometry is widely used in disciplines such as forensic medicine, taxonomy, and zooarchaeology due to certain differences between breeds and genders (Kobryńczuk et al., 2008; Adebisi, 2009; Onar et al., 2015). Polymorphism is very common in sheep breeds, making purely morphological classification difficult. Therefore, morphometric studies are very important for taxonomy (Kaymakçı & Sönmez, 1996; Soysal et al., 2003). Although there are some studies on Akkaraman sheep, as yet there has been no comprehensive anatomic study investigating the morphometric parameters of the skull in Akkaraman and Kangal Akkaraman sheep. The aim of this study was to determine the craniometric data of Akkaraman and Kangal Akkaraman sheep, which are local breeds in Turkey, and to calculate the indexes obtained using craniometric measurements.

MATERIAL and METHODS

Animals

This study used the skulls of 12 male Akkaraman and 12 male Kangal Akkaraman sheep, each aged between 8 and 12 months old. The research materials were collected from private slaughterhouses in the provinces of Konya and Sivas. This study was performed with the permission of Selçuk University Experimental Animal Breeding and Experimental Research Center Ethics Committee (SÜVDAMEK) (decision no: 2022/20).

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Three-dimensional modelling of the images and performing their craniometric measurements

A 64-slice CT scanner (Toshiba Aquillon CX-Tokio/Japan) was used to scan the sheep skulls, and the images obtained were stored in DICOM format. They were then uploaded to the three-dimensional modeling application, 3D Slicer 5.0.3, where 3D models of the skull were formed and the craniometric measurements were recorded. Craniometric measurements were taken of 13 distinct parameters on the 3D models of the skulls. The morphometric measurements were taken based on the measurement sites described in the literature (Von den Driesch, 1976). Osteometric measurements were used to calculate five distinct indices. The formula for the indices generated using these measurement sites was shown in Table 2. The linear measurement points acquired from the dorsal, ventral and lateral surfaces of the skull were shown in Table 1. The research employed Nomina Anatomica Veterinaria for the nomenclature (Nomina, 2017).

Statistical analysis

Statistical analyses of craniometric measurements of skull data were performed in SPSS software version 26 (IBM Corp., Armonk, NY, USA). The conformity of the data to normal distribution was determined using the Shapiro-Wilk test and it was confirmed that the data showed normal distribution. Statistical differences between groups were analyzed using the independent samples t test. Results were expressed as mean ± standard deviation (SD) values. A value of $p<0.05$ was considered statistically significant.

Table1. Studied cranial parameters (cm).

Parameter No	Parameters	Definition
1	Skull length	Akrokranion - Prosthion
2	Greatest length of the nasal bone	Nasion - Rhinion
3	Medial frontal length	Akrokranion - Nasion
4	Greatest breadth across the nasal	Maximum distance across the nasal bones
5	Facial width	Distance between the caudal extents of the orbital rims.
6	Least breadth between the orbits	Entorbitale - Entorbitale
7	Greatest breadth of the Foramen magnum	The maximum distance between the two occipital condyles.
8	Height of the Foramen magnum	Basion - Opisthion
9	Greatest inner length of the orbit	Ectorbitale - Entorbitale
10	Viscerocranium length	Nasion - Prosthion
11	Cranial width	Distance between two external auditory meatus
12	Skull width	Distance between two zygomatic arches
13	Greatest frontal breadth	Ectorbitale - Ectorbitale

Table 2. Indices and formulas of the skulls.

İndex No	Craniofacial İndexes	Formulas
1	Skull index	Skull width/ Skull length× 100
2	Cranial index	Cranial width/ Medial frontal length× 100
3	Nasal index	Greatest breadth across the nasal/ Greatest length of the nasal bone× 100
4	Facial index	Facial width/ Viscerocranium length× 100
5	Foramen magnum index	Height of the Foramen magnum/ Greatest breadth of the Foramen magnum× 100

RESULT

In this study, the first reports of craniometric measurements of sheep from the Akkaraman and Kangal Akkaraman breeds were presented. Craniometric measurements were used to calculate the cranial-facial indices in both species. A total of 13 different craniometric measurements of the skull were made for the investigation (Figure 1). The mean values, stan-

dard deviations, coefficients of variation, maximum and minimum values for each parameter were shown in Table 3, and the derived indexes used in the current investigation on the skull were shown in Table 4.

in only one of the calculated index values, the skull index of the Akkaraman sheep was higher than that of the Kangal Akkaraman sheep.

The mean length and width of the skull were measured as

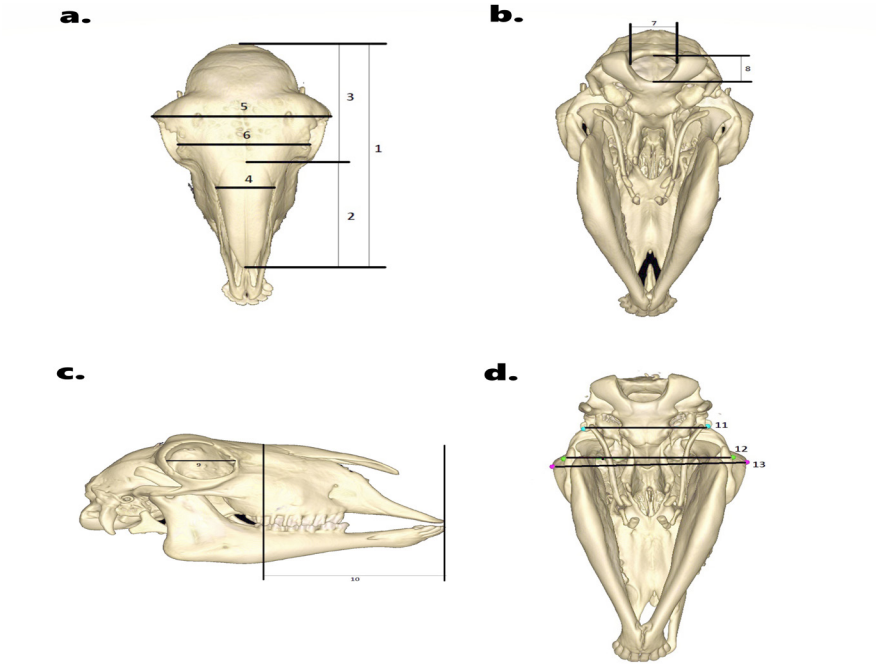

Figure 1. Measurement of the skull of Kangal Akkaraman sheep. (a) Dorsal view, (b,d) Ventral view, (c) Lateral view. 1-Skull length, 2-Greatest length of the nasal bone, 3-Medial frontal length, 4-Greatest breadth across the nasal, 5-Facial width, 6-Least breadth between the orbits, 7-Greatest breadth of the foramen magnum, 8-Height of the foramen magnum, 9-Greatest inner length of the orbit, 10-Viscerocranium length, 11-Cranial width 12-Skull width, 13-Greatest frontal breadth.

All the statistically important data specified in Table 3 should be mentioned in this section in respect of the parameters of skull length, skull width, greatest breadth across the nasal, greatest height of the foramen magnum, greatest frontal breadth, and least breadth between the orbits ($p<0.05$). In terms of the estimated craniofacial indices, the largest inner width of the orbit, and viscerocranium length, no statistically significant difference was determined between the breeds ($p>0.05$) (Table 3 and 4). Based on the metric measurement points, the skull length was found to be the longest measurement, determined as mean 18.37 ± 0.65 cm in Akkaraman sheep and 20.08 ± 0.29 cm in Kangal Akkaraman sheep ($p<0.001$) (Table 3). The foramen magnum height, measured as mean 1.93 ± 0.13 cm in Akkaraman sheep and 2.07 ± 0.16 cm in Kangal Akkaraman sheep ($p=0.039$), was seen to be the smallest measurement. The foramen magnum index had the highest value ($p=0.871$) and the nasal index had the lowest value ($p=0.269$) for the craniofacial index.

DISCUSSION

In this study, 13 craniometric parameters were measured and 5 craniofacial indices were calculated. In all the craniometric measurements, the values of the Akkaraman sheep were lower than those of the Kangal Akkaraman sheep. However, 18.37 ± 0.65 cm and 8.82 ± 0.46 cm, respectively in Akkaraman sheep, and as 20.08 ± 0.29 cm and 9.50 ± 0.34 cm, respectively in Kangal Akkaraman sheep. According to the literature, the skull length of Akkaraman and Kangal Akkaraman sheep was shorter than the skull length of Hasak and Hasmer (Can et al., 2022) Suffolk Down (de la Barra et al., 2020), Morkaraman (Özcan et al., 2010), Xisqueta (Parés Casanova et al., 2010), Awassi (Yılmaz & Demircioğlu, 2020), Bardhoka (Gündemir et al., 2020), Hemshin (Dalga et al., 2018), Hamdani (Dayan et al., 2022), Sharri (Jashari et al., 2022), Iranian Native (Monfared, 2013), and Barbados Black Belly sheep (Mohamed et al., 2016). The skull length of the Akkaraman sheep was shorter than that of the Tuj sheep (Özcan et al., 2010) and longer than that of the Kangal Akkaraman sheep. The skull length of the Kangal Akkaraman sheep was longer than that of male Zell and Mehraban sheep, and the skull length of the Akkaraman sheep was shorter than that of male Zell and Mehraban sheep (Karimi et al., 2011; Marzban Abbasabadi et al., 2020). Moreover, in the current study, it was found that the skull width of Akkaraman and Kangal Akkaraman sheep was lower than Mehraban sheep (Karimi et al., 2011). The skull width of both Akkaraman and Kangal Akkaraman sheep was higher than that of male Zell sheep (Marzban Abbasabadi et al., 2020). It is believed that the differences in skull length and width between breeds of animals are due to the geographical environment in

Table 3. Results of morphometric parameters of the skull of a male Akkaraman sheep and male Kangal Akkaraman sheep determined by 3D reconstruction of CT images.

Parameters (cm)	Groups						P values
	Akkaraman Sheep			Kangal Akkaraman Sheep			
	Mean ± SD	Min-Max	CV	Mean ± SD	Min-Max	CV	
Skull length	18.37 ± 0.65	16.80 - 19.10	3.53	20.08 ± 0.29	19.80 - 20.70	1.42	<0.001
Skull width	8.82 ± 0.46	8.10 - 9.60	5.20	9.50 ± 0.34	9.06 - 10.03	3.62	0.001
Greatest length of the nasal bone	7.62 ± 0.47	6.70 - 8.30	6.20	8.35 ± 0.69	7.10 - 9.80	8.25	0.007
Greatest breadth across the nasal	3.04 ± 0.23	2.80 - 3.40	7.60	3.47 ± 0.18	3.20 - 3.80	5.26	<0.001
Medial frontal length	10.74 ± 0.45	10.10 - 11.40	4.15	11.73 ± 0.67	10.40 - 13.00	5.72	<0.001
Cranial width	5.95 ± 0.36	5.20 - 6.40	6.05	6.61 ± 0.38	6.20 - 7.60	5.75	<0.001
Viscerocranium length	10.03 ± 0.53	9.50 - 11.08	5.32	10.36 ± 1.03	9.50 - 13.30	9.96	0.329
Facial width	10.25 ± 0.37	9.60 - 10.70	3.66	10.77 ± 0.41	10.10 - 11.50	3.84	0.004
Height of the foramen magnum	1.93 ± 0.13	1.70 - 2.20	7.08	2.07 ± 0.16	1.70 - 2.30	8.01	0.039
Greatest breadth of the foramen magnum	2.05 ± 0.13	1.80 - 2.30	6.69	2.21 ± 0.15	2.07 - 2.50	6.87	0.017
Greatest frontal breadth	10.44 ± 0.43	9.80 - 11.30	4.18	11.04 ± 0.33	10.50 - 11.50	3.04	0.001
Greatest inner length of the orbit	3.72 ± 0.15	3.40 - 3.90	4.14	3.80 ± 0.22	3.40 - 4.10	5.91	0.351
Least breadth between the orbits	7.47 ± 0.51	6.40 - 8.10	6.83	7.86 ± 0.31	7.30 - 8.40	3.99	0.034

which they are raised, their care and feeding conditions, the calcium ratio in their feed, and breed-specific characteristics.

The current study showed that the medial frontal length of Akkaraman and Kangal Akkaraman sheep was shorter than that of Xisqueta (Parés Casanova et al., 2010), Mehraban (Karimi et al., 2011), Iranian Native (Monfared, 2013), and Barbados Black Belly sheep (Mohamed et al., 2016), but the medial frontal length of Kangal Akkaraman sheep was almost equal to that of Iranian Native sheep (Monfared, 2013). Although the medial frontal length of Akkaraman sheep and Tuj sheep were the same, the medial frontal length of Kangal Akkaraman sheep was greater than that of Morkaraman and Tuj sheep (Özcan et al., 2010).

In the present study, the greatest breadth across the nasal

Table 4. The results of indexes of skull of male Akkaraman sheep and male Kangal Akkaraman sheep obtained through 3D reconstruction of CT images.

Index (%)	Groups						P values
	Akkaraman Sheep			Kangal Akkaraman Sheep			
	Mean ± SD	Min-Max	CV	Mean ± SD	Min-Max	CV	
Skull index	48.06 ± 2.33	44.92 – 52.17	4.85	47.31 ± 1.86	43.96 – 49.49	3.93	0.391
Cranial index	55.41 ± 3.01	49.56 – 59.43	5.43	56.52 ± 4.05	49.23 – 66.09	7.17	0.455
Nasal index	39.97 ± 3.23	34.57 – 47.76	8.08	41.83 ± 4.66	32.65 – 52.11	11.16	0.269
Facial index	102.46 ± 6.92	93.20 – 111.58	6.76	104.74 ± 10.83	79.70 – 121.05	10.34	0.545
Foramen magnum index	106.92 ± 10.11	94.74 – 122.22	9.46	107.76 ± 14.71	94.09 – 141.18	13.65	0.871

P value is indicated in bold for the parameters with statistical differences in the data analyzed using the independent sample t-test. Abbreviation: CV, Coefficient of variations.

was greater than that of Mehraban (Karimi et al., 2011), Morkaraman and Tuj sheep (Özcan et al., 2010). Moreover, greatest length of the nasal bone in Akkaraman and Kangal Akkaraman sheep was shorter than that of Xisqueta (Parés Casanova et al., 2010), Sharri (Jashari et al., 2022), Suffolk Down (de la Barra et al., 2020), Iranian Native (Monfared, 2013) and Mehraban sheep (Karimi et al., 2011). Akkaraman and Kangal Akkaraman sheep had higher values than Morkaraman and Tuj sheep (Özcan et al., 2010). The greatest length of the nasal bone of Kangal Akkaraman sheep was longer than that of Barbados Black Belly (Mohamed et al., 2016), Akkaraman and male Zell sheep (Marzban Abbasabadi et al., 2020). However, the greatest value of nasal bone length of Akkaraman sheep was higher than that of male Zell sheep (Marzban Abbasabadi et al., 2020).

Facial width and viscerocranium length were 10.25 ± 0.37 cm and 10.03 ± 0.53 cm, respectively in Akkaraman sheep, and 10.77 ± 0.41 cm and 10.36 ± 1.03 cm, respectively in Kangal Akkaraman sheep. The viscerocranium length of Akkaraman and Kangal Akkaraman sheep was shorter than that of Xisqueta (Parés Casanova et al., 2010), Mehraban (Karimi et al., 2011), Morkaraman, and Tuj sheep (Özcan et al., 2010). These results show that the viscerocranium length of Akkaraman and Kangal Akkaraman sheep was higher than that of male Zell sheep (Marzban Abbasabadi et al., 2020). The facial width was almost the same in Akkaraman, Kangal Akkaraman, and Mehraban sheep (Karimi et al., 2011). However, the value was higher in Akkaraman and Kangal Akkaraman sheep than in Suffolk Down (de la Barra et al., 2020) and male Zell sheep (Marzban Abbasabadi et al., 2020), but lower than in Sharri sheep (Jashari et al., 2022).

The least breadth between the orbits (entorbitale - entorbitale) was measured as mean 7.47 ± 0.51 cm in Akkaraman sheep, and as 7.86 ± 0.31 cm in Kangal Akkaraman sheep. This parameter in both Akkaraman and Kangal Akkaraman sheep was seen to be higher than in Morkaraman sheep and Tuj sheep (Özcan et al., 2010), but lower than in Sharri (Jashari et al., 2022) and Xisqueta sheep (Parés Casanova et al., 2010).

The distance between the two zygomatic areas in dogs and camels has been reported as the largest area of the skull. Due to morphological differences, the largest area of the sheep skull has been shown to be the parameter of the greatest frontal breadth (ectorbitale - ectorbitale). This value in the current study was determined to be mean 10.44 ± 0.43 cm in the Akkaraman sheep and 11.04 ± 0.33 cm in the Kangal Akkaraman sheep. According to previously reported data, the greatest frontal breadth of both the Akkaraman and Kangal Akkaraman sheep was less than that of Xisqueta (Parés Casanova et al., 2010) and Awassi sheep (Yılmaz & Demircioğlu, 2020), but greater than that of Morkaraman and Tuj sheep (Özcan et al., 2010).

The greatest inner length of the orbit, also known as the orbital width, was measured as 3.72 ± 0.15 cm in Akkaraman sheep and 3.80 ± 0.22 cm in Kangal Akkaraman sheep. This value was lower in Akkaraman and Kangal Akkaraman sheep compared to Suffolk Down (de la Barra et al., 2020), Sharri (Jashari et al., 2022), Xisqueta (Parés Casanova et al., 2010), and Mehraban sheep (Karimi et al., 2011), but higher than in male Zell (Marzban Abbasabadi et al., 2020), Morkaraman and Tuj sheep (Özcan et al., 2010).

The height of the foramen magnum and greatest breadth of the foramen magnum were 1.93 ± 0.13 cm and 2.05 ± 0.13 cm, respectively in Akkaraman sheep, and 2.07 ± 0.16 cm and 2.21 ± 0.15 cm, respectively in Kangal Akkaraman sheep. The height and width values of the foramen magnum were greater in Akkaraman and Kangal Akkaraman sheep than in Xisqueta (Parés Casanova et al., 2010), male Zell (Marzban Abbasabadi et al., 2020) and Mehraban sheep (Karimi et al., 2011), but lower than in Awassi sheep (Yılmaz & Demircioğlu, 2020).

The height and width of the foramen magnum were higher in Kangal Akkaraman sheep than in Morkaraman and Tuj sheep (Özcan et al., 2010). However, the height of the foramen magnum in Akkaraman sheep was lower than in Morkaraman sheep and higher than in Tuj sheep (Özcan et al., 2010), whereas the maximum breadth of the foramen magnum was lower in Akkaraman sheep than in Morkaraman and Tuj sheep (Özcan et al., 2010). Based on the values obtained in the study, the height of the foramen magnum was higher in Akkaraman and Kangal Akkaraman sheep than in Sharri (Jashari et al., 2022) and Suffolk Down sheep (de la Barra et al., 2020), but the greatest breadth of the foramen magnum was lower than in Sharri (Jashari et al., 2022) and Suffolk Down sheep (de la Barra et al., 2020).

Skull index values are used in bone deformities and in the evaluation of brain development. The index values of sheep breeds are important in determining the typology of breeds (Kanchan et al., 2014; Onar & Pazvant, 2001). The skull index of the Akkaraman sheep was found to be 48.06 ± 2.33 cm. This index was higher in Akkaraman and Kangal Akkaraman sheep than in Sharri sheep (Jashari et al., 2022), Xisqueta sheep (Parés Casanova et al., 2010), and Awassi sheep (Yılmaz & Demircioğlu, 2020), but lower than in Hemshin sheep (Dalga et al., 2018), Morkaraman and Tuj sheep (Özcan et al., 2010). However, it was determined that the skull index of Akkaraman and Kangal Akkaraman sheep was lower than that of Mehraban sheep (Karimi et al., 2011), Morkaraman, and Tuj sheep (Özcan et al., 2010).

The nasal and facial index values of the Akkaraman and Kangal Akkaraman sheep in this study were higher than those of Morkaraman and Tuj sheep (Özcan et al., 2010). Similarly, the facial index values of the Akkaraman and Kangal Akkaraman sheep were higher than those of the Xisqueta sheep (Parés Casanova et al., 2010), Sharri sheep (Jashari et al., 2022), Hemshin sheep (Dalga et al., 2018), and Mehraban sheep (Karimi et al., 2011).

The value of the foramen magnum index in this study was determined to be mean 106.92 ± 10.11 in Akkaraman sheep and 107.76 ± 14.71 in Kangal Akkaraman sheep, which may be attributed to the fact that the width of the foramen magnum is greater than the height. The differences between these values may be due to breed differences and, in part, to differences in the measurement methods used.

CONCLUSION

In conclusion, it is thought that the results of this study will serve as a reference for science branches such as osteoarchaeology, anatomy, for the creation of a craniometric measurement and index database for Akkaraman and Kangal Akkaraman sheep, and it can also be used for taxonomic classification of species in Turkey and breeding ram determination. Nevertheless, further studies with larger sample sizes could produce more precise results in the measured parameters.

DECLARATIONS

Ethics Approval

This study was performed with the permission of the Selçuk University Experimental Animal Breeding and Experimental Research Center Ethics Committee (SÜVDAMEK) (decision no: 2022/20).

Conflict of Interest

All the authors have read and approved the manuscript. The authors have no conflict of interests to declare.

Consent for Publication

Not applicable

Author contributions

Idea, concept, and design: HBE, KB, NB

Data collection and analysis: HBE

Drafting of the manuscript: HBE, KB

Critical review: HBE, KB, NB

Data Availability

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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This study is a part of the doctoral thesis of the first author.

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Research Article

Evaluation of eye diseases in cats and dogs: A retrospective study: 200 cases (2021-2022)

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ABSTRACT

In this study, the incidence of ocular diseases in cats and dogs brought to Burdur Mehmet Akif Ersoy University Animal Hospital Surgery Clinic between 2021-2022 was investigated. The material of study consisted of 200 patients (103 dogs and 97 cats). Ocular pathology was diagnosed in 35.05% of the examined cats and 35.92% of the examined dogs. The most common cat breed with ocular pathologies was mixed breeds with a rate of 29.8%, and the most common dog breed was Golden Retrievers with a rate of 82.3%. When the anatomical localization of the pathologies encountered in the cats and dogs included in the study was evaluated, it was seen that the most common anatomical region with pathologies in cats was the cornea, and the most common anatomical region with pathologies in dogs was the lens. According to the data recorded this study the study, the most common ocular pathologies were corneal damage 14.7% for cats, and senile nuclear sclerosis 36.75% for dogs. In conclusion, this study aimed to help veterinarians to approach ocular diseases by identifying the most common ocular diseases in cats and dogs.

INTRODUCTION

The eye is the organ that has undertaken an important function such as vision and is the most sensitive to external factors and diseases (Kahn, 2007). Ocular diseases form an important part of small animal practice (Scountzou, 2003). The occurrence of ocular diseases may be caused by physical effects such as traumas, infectious and metabolic factors (Akın & Samsar, 2005). Congenital or acquired ocular and palpebral diseases are frequently encountered in cats and dogs (Glaze, 1997; Narfstrom 1999). It can also be seen in congenitally developing disorders specific to species and breeds (Akın & Samsar, 2005). In some cases, systemic diseases can be clinically reflected in the eye. Early and prominent ocular findings can be observed in all infectious, neoplastic, autoimmune, nutritional, toxic, and metabolic diseases (Ollivier et al., 2013; Schaer et al., 2006).

The eye is very sensitive organ, the function of which may be affected even with a mild insult to its homeostasis, due to direct injury or other local or systemic diseases and studies on ocular affections may provide information on the prevalence of ocular diseases and also help to limit diagnostic possibilities and treatment options (Kumar et al., 2018).

In this study, the ocular diseases of 97 cats and 103 dogs brought to Burdur Mehmet Akif Ersoy University Veterinary Faculty Animal Hospital Surgery Clinic between 2021-2022 were evaluated retrospectively.

MATERIAL and METHODS

The material of this study consisted of 97 cats and 103 dogs brought to Burdur Mehmet Akif Ersoy University Animal Hospital Surgery Clinic between 2021-2022. Routine eye examinations were performed after obtaining the anamnesis of all cases. In addition to a routine ocular examination, Schirmer tear test and fluorescein test were used when necessary. As a result of routine ocular examinations, the incidence of healthy and pathological cases was determined on the basis of species. While evaluating the pathological cases, the breeds in which the pathology was seen most frequently, the frequency and localization of the lesion on the basis of breed were determined. The pathological cases encountered as a result of the examinations made for this purpose were recorded on the basis of species and their photographs were taken.

RESULTS

In this study, 97 cats and 103 dogs brought to Burdur Mehmet Akif Ersoy University Animal Hospital Surgery Clinic between 2021-2022 were examined. The recorded pathologies and breeds were converted into data by calculating their values in the percentile system. Ocular pathology was diagnosed in 35.05% of the examined cats and 35.92% of the examined dogs (Figure 6, Figure 7). The cat breed with the most ocular pathologies was mixed breed with a rate of 29.8% (Figure 8). The dog breed with the most ocular pathologies was Golden Retrievers with a rate of 82.3% (Figure 9). Ocular disease diagnosed in cats were recorded as corneal necrosis, synechia, blepherospasm, heterochromia, herpes virus infection, uveal melenoma, symblepharon, lens luxation, uveitis, bacterial in-

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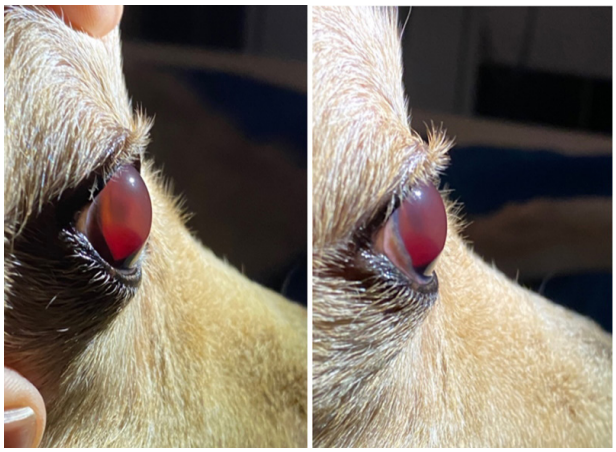

Figure 1. A case of hyphema caused by neoplasia in a dog.

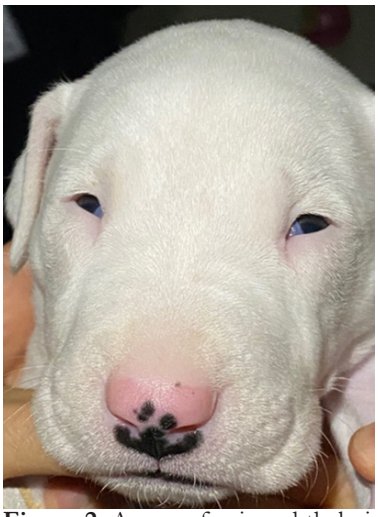

Figure 2. A case of microphthalmia in a dog.

Figure 3. A case of fluorescein positive corneal necrosis in a cat.

fection, hyphema, descemetocele hernia, cartilage eversion, entropion, anisocoria, corneal damage, foreign body, corneal ulcer (Figure 3, Figure 4, Figure 5). Ocular disease diagnosed in dogs were recorded as senile nuclear sclerosis (SNS), cataract, corneal damage, microphthalmia, papilloma, hyphema, glaucoma, corneal edema, neoplasia, heterochromia, scleral hemorrhage, and uveitis (Figure 1, Figure 2). The anatomical localization of the pathologies encountered in the cats and

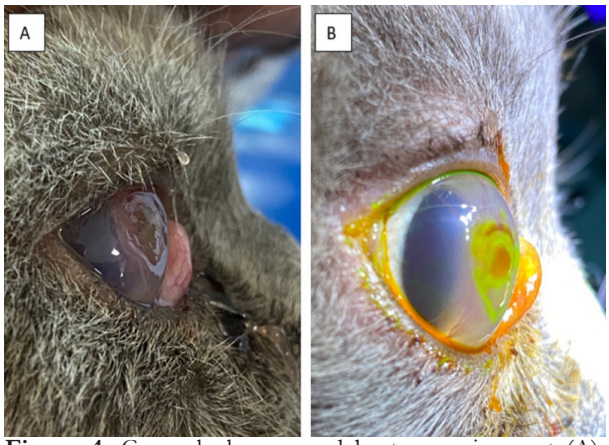

Figure 4. Corneal ulcer caused by trauma in a cat (A), fluorescein dye positive appearance (B).

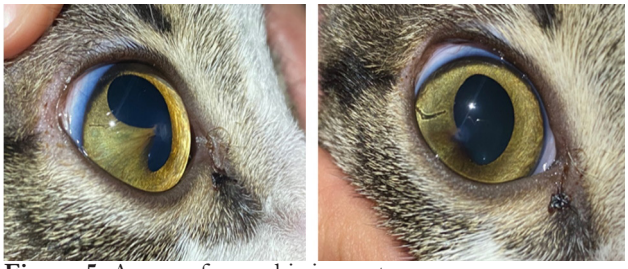

Figure 5. A case of synechia in a cat.

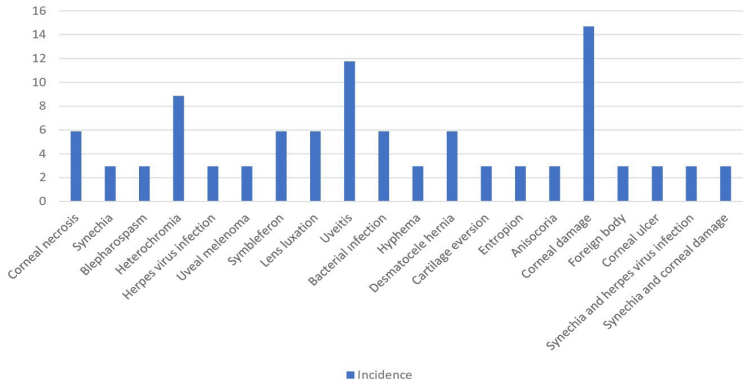

Figure 6. The percentage incidence of eye pathologies recorded in cats is shown in the graphic.

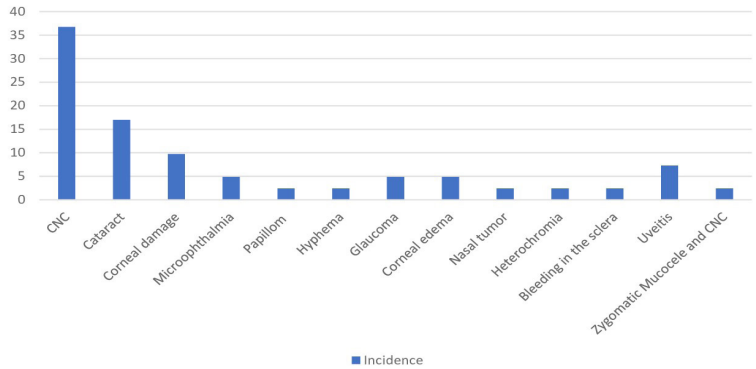

Figure 7. The percentage of occurrence of eye pathologies recorded in dogs is shown in the graphic.

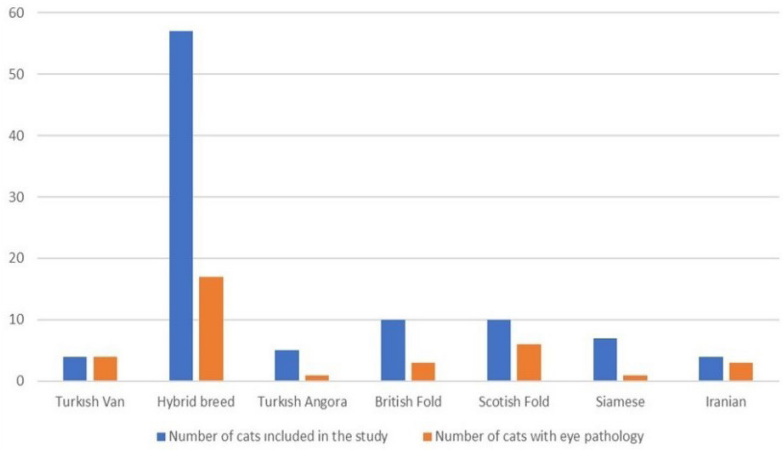

Figure 8. Cat breeds (Blue) included in the study and the number of cats with eye pathology (Orange) by breed are shown in the graphic.

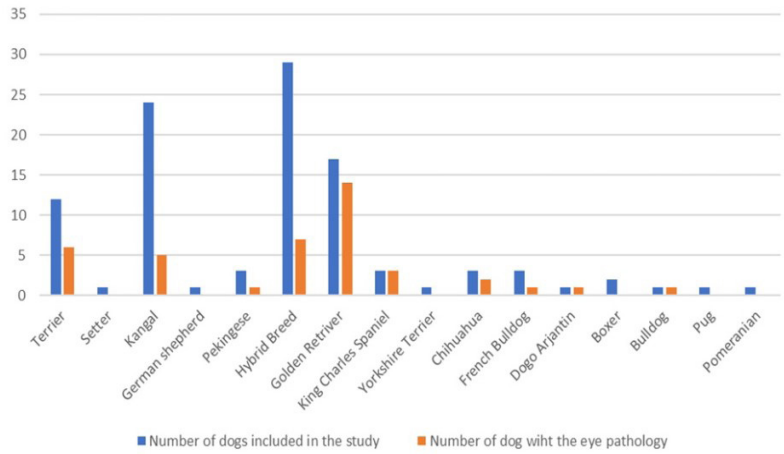

Figure 9. The dog breeds (Blue) included in the study and the number of dogs with eye pathology (Orange) by breed are shown in the graphic.

dogs included in the study was evaluated, it was seen that the most common anatomical region with pathologies in cats was the cornea, and in dogs was the lens. The most frequently diagnosed ocular disease in cats was recorded as corneal damage with rate of 14.7%, and in dogs was recorded as SNS with a rate of 36.75%.

DISCUSSION

Akinrinmade and Ogungbenro (2015), reported a total of 231 cases of ocular disease out of a total of 3,488 cases examined in their study. Kumar et al. (2018), reported a total of 4500 dog clinical cases with different clinical effects over a period of more than one year, and 60 dogs were brought with ophthalmological complaints by their owners. Han et al. (2019), examined 278 animals from 9 species (cat, dog, cattle, sheep, goat, horse, bird, turtle, rabbit) with ocular disease. Deveci et al. (2020), evaluated 30 ocular and palpebral diseases of different localization and type in a total of 201 cases in cats and dogs (64 cats and 137 dogs). Uzunlu et al. (2020), diagnosed ocular disease in 78 (46.43%) cats and 90 (53.57%) dogs out of 5000 patients in their study. In this study, 200 cases (97 cats and 103 dogs) brought to Burdur Mehmet Akif Ersoy University Animal Hospital Surgery Clinic between 2021-2022 were examined.

Kumar et al. (2018), found the incidence of ocular involvement in dogs to be 1.33%. In this study, ocular pathology was diagnosed in 35.05% of the examined cats and 35.92% of the examined dogs. Kumar et al. (2018), when they evaluated the distribution of cases by breed, Pugs (28.3%) showed the highest prevalence of ocular diseases, followed by mixed breeds (21.7%) and Labrador Retriever (20%). Ocular affections were also reported in other breeds like Spitz (15%), German Shepherd Dog (11.7%) whereas Bully breed had the least incidence (3.3%) of ocular affections. Akinrinmade and Ogungbenro (2015), evaluated breeds with ocular disease in their study, they found that the Alsatian breed was the most affected (22.08%), while other breeds such as Toy breed, Rottweiler, Boerboel and Mongrel were equally and moderately affected. They identified the Caucasian breed as the breed with the lowest incidence (2.16%). Uzunlu et al. (2020), revealed that the dog breed with the most ocular disease was the mixed breed (34.44%), and the cat breed with the most common ocular disease was the mixed breed (93.59%). In the study, the cat breeds with the most common ocular disease were mixed breeds with a rate of

29.8%, while the dog breed with the most ocular disease was the Golden Retriever breed with a rate of 82.3%.

Akinrinmade and Ogungbenro (2015), classified ocular diseases anatomically in their study, they were observed that the highest involvement occurred in the palpebra and/or conjunctiva (58.01%) and the least involvement in the lens (22.51%) and cornea (19.48%) occurred. Gültekin (2020), revealed the corneal lesions (48.3%) are the most common ocular diseases in brachiocephalic breeds. Han et al (2019), reported that the most commonly affected anatomical regions were the cornea (39.93%), conjunctiva (36.7%), retina (6.48%), palpebra (5.8%). When the anatomical localization of the pathologies encountered in the cats and dogs included in this study was evaluated, in cats was the cornea, and in dogs was the lens.

Kumar et al. (2015), reported the most observed ocular diseases as pigmentary keratitis/keratoconjunctivitis (21.7%), corneal ulcer/injury (21.7%), corneal opacity (18.3%), epiphora (11.6%), and cloudy eye (8.3%). Deveci et al (2020), reported that the most common ocular diseases were conjunctivitis (17.91%) and glandula nictitans prolapse (11.94%). Han et al. (2019), reported that the most common ocular diseases in all 278 cases were keratitis (34.17%) and conjunctivitis (31.29%). In a study conducted by Pandey et al. (2018), on the incidence of ocular diseases in dogs, they stated that the cataract in dogs. In this study, the ocular pathologies diagnosed in these cats are corneal necrosis, synechia, blepherospasm, heterochromia, herpes virus infection, uveal melenoma, symbleferon, lens luxation, uveitis, bacterial infection, hyphema, descemetocele hernia, cartilage eversion, entropion, anisocoria, foreign body damage, and corneal ulcer. The ocular pathologies diagnosed in dogs were SNS, cataract, corneal damage, microphthalmia, papilloma, hyphema, glaucoma, corneal edema, neoplasia, heterochromia, scleral hemorrhage, and uveitis. When the incidence of ocular pathologies diagnosed in cats was examined, corneal damage was recorded as 14.7%, and SNS was the most common pathological case in dogs with a rate of 36.75%.

In a study of 47 dogs with palpebral tumors in dogs, the most common palpebral tumor was found to be meibomian epithelioma (36.17%), and papilloma was observed in 1 dog (2.12%) (Kaya et al., 2018). In this study, the incidence of papilloma in dogs was recorded as 2.43%.

CONCLUSION

As a result; in this study, a retrospective evaluation of 200 cases in cats and dogs with various ocular and palpebral diseases is presented in this study. It has been determined that many of the ocular and palpebral diseases are more common in cats than in dogs.

DECLARATIONS

Ethics Approval

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

Consent for Publication

Not applicable.

Author Contributions

Idea, concept and design: ÖŞŞ, MNÇ

Data collection and analysis: ÖŞŞ, MNÇ, BN

Outline of the article: ÖŞŞ, MNÇ, BN

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The author has provided the required data availability statement, and if applicable, included functional and accurate links to said data therein.

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Research Article

An investigation of West Nile virus (WNV) infection in local wild birds species

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ABSTRACT

The transmission of West Nile Virus (WNV) to new locations is mostly facilitated by migratory birds. Türkiye's domestic ducks, geese, and chickens have already tested positive for WNV by serology. This study was conducted to identify the seroprevalence of WNV in wild bird species because wild birds in the Western Mediterranean Region are found along migration routes from Africa to Europe, they are home to a wide variety of bird species due to the abundance of lakes and wetlands, the mild Mediterranean climate, and some areas that are suitable habitat for mosquitoes due to their low altitude. Serum samples were taken from 141 wild birds in Isparta (66), Burdur (42), and Antalya (33) for this study on birds in the wild. During serological studies, there was no evidence of WNV-specific antibodies.

INTRODUCTION

The RNA virus known as West Nile Virus (WNV) belongs to the Flaviviridae family. Antigenically, it is also very similar to the dengue and yellow fever viruses. The substance is membrane-bound, single-chained, icosahedral, positively polarized, and between 40 and 60 nm in size (Devine, 2003). WNV has ten serological subgroups. The RNA genome of WNV is coded by three structural and seven non-structural proteins. Virion creation requires structural proteins, but immunological infiltration and viral transcription and replication require non-structural proteins (Kramer et al., 2008; Kireçci et al., 2011). The West Nile region of Uganda is where WNV was initially discovered in 1937. In addition to Africa, this agent was found in Asia, the United States, the Southern and Eastern areas of Europe, Australia (Marfin & Gubler, 2001) and Türkiye (Kalaycioglu et al., 2012). Though bloodsucking flies of the *Culex and Aedes* species are the disease's primary carriers, tick infection has also been documented. The hosts on which the virus is reproduced are birds. The last two hosts are thought to be horses and people. The WNV titer is quite high in wild bird blood. Birds that migrate have a part in spreading the virus to new areas. The majority of hosts on whom the virus normally replicates are birds. One of the main causes of this is that reservoir birds may produce significant amounts of viremia (Hayes et al., 2005; Uyar & Bakır, 2016). This infectious agent is typically found in avian species and is spread by mosquitoes to people and domesticated mammals. As a result, however infrequently, domesticated creatures and humans may exhibit diseases and mortality cases. WNV infection is typically found in wetlands locations with high mosquito populations, warm and hot temperature zones, and migratory birds may also carry it. With this work, we sought to identify the serological evidence of WNV infection in a number of wild bird species found in the Western Mediterranean region.

MATERIAL and METHODS

Animals and Sample collection

In this study, 0.5-1 ml blood was collected from veins on the inner wing surfaces of wild birds in, Isparta SDU Faculty of Agriculture Research Farm (66) Antalya Zoo (33) and Burdur-Karakent village Lisinia Nature Rehabilitation Center (19). Isparta, Antalya, Burdur, coordinates, respectively (30 E 33, 37 N 46), (30 E 42, 36 N 54), (30 E 17, 37 N 43). The following tools were employed to achieve this goal: a disposable sterile tuberculin needle and injector, alcohol, betadine disinfectant, sterile adhesive bandage, and disposable sterile gloves. Throu-

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gh the use of a sterile injector, blood samples were obtained from the hearts of dead animals.

Figure 1. Locations of serum samples collected from Southwest region of Türkiye.

The collected blood samples were put into sterile blood tubes and sent to the lab with a +4 °C cold chain. Blood samples were centrifuged at 2000 rpm for 20 minutes. The collected serum samples were then placed in eppendorf tubes and stored at a low temperature (-20 °C) until testing.

Indirect ELISA

Blood samples were brought to room temperature after being solubilized in a bain marie. During testing, the WNV-ELISA (Cat No. WNC-2P) (antibody) test kit from ID-Vet (France) company was utilized. The testing was done in accordance with the company's protocol. The test findings were used in the calculation of the test kit and were either detected as positive, suspicious, or negative.

RESULTS

At the conclusion of this investigation, no local wild bird species in Türkiye's Western Mediterranean region (Burdur, Isparta, and Antalya) had been shown to have WNV antibodies (Table 1). During testing, no questionable samples were discovered.

DISCUSSION

According to a report by the OIE (2018), WNV was thought to have been a disease discovered in humans in Africa around the 1950s (2018). Prior to 1996, encephalitis-progressing WNV cases were noted in a number of residents of France, Greece, Israel, Italy, North America, Romania, Russia, and Tunisia. In the 1960s, WNV infection was initially discovered in horses in Egypt and France (Schmidt & Mansoury, 1963). The infection was discovered in horses in the following years in the USA, Spain, Italy, France, Israel, Morocco, Canada, and Argentina (Frost et al., 2012). Antibodies against WNV have been found in humans, animals, and livestock in the Mediterranean and Aegean regions (Ozkul et al., 2006). These include sheep, cattle, dogs, horses, donkeys, and mules. Albayrak & Ozan (2013) were unable to find specific antibodies against WNV in sera of horses, sheep, cattle, and buffaloes gathered from various places in the Black Sea region.

Birds and mosquitoes of the Culex sp. species, which are involved in the virus's biological cycle, are important in the transmission of WNV infection. There was a significant incidence among birds, including geese, chickens, pigeons, and swallows. However, it was discovered that the virus had spread without the aid of vectors in a study on migratory geese. In both horses and people, the virus does not replicate (Erdem & Pahsa, 2003; Austin et al., 2004). Within their habitats, mosquitoes deposit their eggs in quiet, muddy waterways. They also use the blood of migrating birds that live in these locations to sustain themselves. They spread a virus through their saliva to the birds they feed on while they are doing so, which results in a protracted period of viremia. Migratory birds are a crucial reservoir for spreading the virus from one region to another (Hayes et al., 2005).

In particular, epidemics were observed as migrating birds flew from Africa to Europe. Because they travel greater distances, viremic migratory birds spread the agent more widely than domestic birds do. When birds are migrating and there are a lot of mosquitoes, which is towards the conclusion of the summer season and the beginning of fall, WNV epidemics appear (Hayes et al., 2005; Malkinson et al., 2002). Türkiye is situated along the bird migration pathways that connect Africa and Europe. The Western Mediterranean region, sometimes referred to as the "Lake District" is one of the most significant stops for migrating birds. Four separate locations in the Western Mediterranean region saw wild bird samplings. Migrational birds that had been injured while being hunted provided samples from the province of Burdur to the veterinary faculty clinics. In terms of species diversity, the zoo in Antalya that houses wild birds is significant to us. These gathered samples were thought to be crucial in identifying WNV seroprevalence.

ELISA, such as hemoagglutinin inhibition (HI), Virus Neutralization (VN), and Plaque Reduction Neutralization assays are used to determine the seroprevalence of WNV in birds (OIE, 2018). Yapici et al. (2012) used the ELISA test to identify serological signs of WNV infection in birds and thought it was a quick, easy, and accurate method. According to Padilla et al. (2009), the ELISA test's specificity was 99.4% and its sensitivity was 84.9%. Domestic chickens in the Konya province were not seropositive for WNV, according to Yapici

Table 1. WNV antibody results for wild birds in Western Mediterranean region.

Species	Numbers	WNV ELISA (Ab)
Gold Phoenix (*Phoenix*)	1	Negative
Ruddy shelduck (*Tadorna ferruginea*)	1	Negative
Brahma (*Gallus gallus*)	3	Negative
Pekin cochin (*Gallus gallus*)	3	Negative
Giant cochin (*Gallus gallus*)	3	Negative
Common pochard (*Aythya ferina*)	2	Negative
American flamingo (*Phoenicopterus ruber*)	1	Negative
Polish chicken (*Gallus gallus*)	1	Negative
Silver pheasant (*Lophura nycthemera*)	1	Negative
Common pheasant (Phasianus colchicus)	17	Negative
Lady Amherst's Pheasant (*Chrysolopus amherstiae*)	1	Negative
Japanase chicken (*Gallus gallus*)	1	Negative
Lesser black-backed gull (*Larus fuscus*)	1	Negative
Rook (*Corvus frugilegus*)	1	Negative
Rock dove (*Columba livia*)	7	Negative
Chukar partridge (*Alectoris chukar*)	61	Negative
Long-legged buzzard (*Buteo rufinus*)	2	Negative
Reeves's pheasant (*Syrmaticus reevesii*)	1	Negative
Little owl (*Athena noctua*)	2	Negative
Eurasian collared dove (*Streptopelia decaocto*)	1	Negative
White stork (*Ciconia ciconia*)	8	Negative
Budgerigar (*Melopsittacus undulatus*)	1	Negative
Muscovy duck (*Cairina moschata*)	1	Negative
Pekin duck (*Anas platyrhynchos*)	1	Negative
Great white pelican (*Pelecanus onocrotalus*)	1	Negative
Common starling (*Stumus vulgaris*)	1	Negative
Zibrit golden chicken (*Gallus gallus*)	1	Negative
Dead sea sparrow (*Passer moabiticus*)	3	Negative
Common buzzard (*Buteo buteo*)	5	Negative
Indian peafowl (*Pavo cristatus*)	5	Negative
Wild turkey (Meleagris gallopavo)	1	Negative
Green pheasant (*Phasianus versicolor*)	1	Negative
Mallard duck (*Anas platyrhynchos*)	1	Negative
TOTAL	**141**	

et al (2012) .'s research. In his investigation, Pir (2016) found that 4.3% of domestic birds had antibodies against WNV. According to him, high seropositivity zones are found around the Kızılırmak river and may be brought on by migrating birds passing through the delta. In their research, Ergünay et al. (2014) discovered a 9.9% seropositivity rate for WNV in ducks near the province of Kars. It was found to be 1.1% (4/378) in geese near Türkiye's northeast by Yildirim et al. (2018). WNV seropositivity rates were reported to be 3.1% for hens, 0.8% for ducks, 1.8% for geese, and 17.9% for turkeys by Pir and Albayrak (2017). 32 out of 736 serum samples were discovered to be positive (4.3%) for WNV antibodies. Out of 155 bird blood serum samples taken in Malaysia, WNV IgG ELISA was used to detect seropositivity in 30 samples (27 migratory birds and 2 indigenous water birds). They claimed that mosquitoes may be connected to WNV illnesses reported in local and migratory aquatic birds (Ain-Najwa et al. 2020).

According to estimates, migratory birds are one of the main vectors of WNV transmission into previously uninfected nations (Rappole et al., 2000). Additionally, the WNV viremia time in investigations on several bird species was no longer than a week (del Amo et al., 2014). Due to the fact that ELISA-positive birds had not recently been infected to WNV and were therefore unlikely to be at the viremia stage, neutralized antibody testing on sera was not conducted on them (Jourdain et al., 2011). For instance, it may take about 12 days for wild birds to migrate from Spain to Germany and Holland. Under these

circumstances, the migratory birds may contract a fly-borne illness cycle, and since the stress of the migration does not affect the duration of the viremia phase, the disease may not spread (Chevallier et al., 2010).

Numerous bird species are susceptible to WNV infection, which can develop and manifest as a variety of clinical signs. Although some animals are immune to the virus, others can have MSS flaws. Domestic goose fatalities and abnormalities have been documented in Canada and Israel. Infection with WNV is fatal to wild birds in Europe (Zeller & Schuffenecker, 2004). White storks and domestic geese showed signs of encephalitis and paralysis during the WNV outbreak in Israel (Malkinson et al., 2002). Many domestic and wild birds were found to have WNV infection in an American zoo (Austin et al., 2004; Steele et al., 2000). In this study, samples from the province of Isparta, Burdur and Antalya antibodies against WNV could not be found. Similarly, in more than 4000 blood serum samples taken from 3300 wild birds, Balança et al. (2009) found a seropositivity rate of fewer than 1%.

According to an OIE report from 1999, domestic geese in Israel had a 27% prevalence of WNV infection. Geese 60 to 70 days old may exhibit clinically detectable central nervous system abnormalities (Swayne & Spackman, 2013). However, because they do not frequently come into contact with flies, birds raised indoors are less prone to contract the disease. Chickens and turkeys are not clinically affected by WNV (OIE, 2000). Similar to this, Calle (2000) reported that out of 277 birds of 74 species kept indoors, WNV seropositivity was not found in 36 of them. Therefore, the fact that we were unable to identify seropositivity in samples obtained from the SDU Agricultural Faculty Research Farm may be related to the fact that these animals are cared for, fed, and housed indoors.

CONCLUSIONS

When WNV infection is discovered in wild bird species, people and domestic mammals may also be at risk. Our nation offers a suitable habitat for WNV hosts and vectors to survive, similar to arboviral infections, in terms of geography and climate. The illness can also be widely prevalent in the nations that are next to us. Based on the findings of this study conducted in the Western Mediterranean region, which is on the migratory routes of migrating birds and features lakes and wetlands, we advise conducting additional serosurvey investigations in densely populated animal groups.

DECLARATION

Ethics Approval

This study was approved by Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee at the meeting dated 20.06.2013 with the number of 36 decisions.

Conflict of Interest

The authors declared that there are no conflicts of interest.

Consent for Publication

For this study, a permit warrant has been taken by the article named as Research Permit from TR Ministry of Forestry and Water Affairs, Nature Reserve and General Directorate for National Parks dated 21st August, 2013 and numbered 72784983-488.04-156205.

Author Contribution

Idea, concept and design: MK, NM

Data collection and analysis: MK, NM, KA, AA

Drafting of the manuscript: YY, HSS, YSA

Critical review: MK, KA, SH, OB

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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