ISSN: 2146-7269
e-ISSN: 2687-5330

ANIMAL HEALTH, PRODUCTION AND HYGIENE

Volume 11, Issue 2 July - December 2022 Page: 1-71

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ANIMAL HEALTH, PRODUCTION AND HYGIENE

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www.dergipark.or.tr/tr/pub/aduveterinary
Animal Health, Production and Hygiene (Animal Health, Prod and Hyg) is publication of Faculty of Veterinary Medicine, University of Aydın Adnan Menderes University. The journal publishes original researches and reviews on all aspects of veterinary science involving farm and pet animals, laboratory, marine and exotic/wild animals, zoonozes and public health.

Owner on behalf of the Faculty of Veterinary Medicine (İmtiyaz Sahibi)
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Research Article

The Use of Demountable Dog Skeleton Model in Osteology Education

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ABSTRACT

The aim of the study is by making the osteology information given in the theoretical part of the anatomy course more understandable in the practical courses; to increase the effectiveness of osteology education. Three dog cadavers were used as animal material. The bones of the cadavers were cleaned with the maceration method. Holes were drilled on the articular surface of the bones and powerful magnets were embedded in such a way that they were facing each other. Magnets were placed between the head and mandible, C1-C6 vertebrae in the anterior and posterior limb joints. Thus, the demountable skeleton was obtained. The possible contributions of these skeletons to the learning of osteology were evaluated by considering the questionnaire, quiz, midterm exam, end-of-year exam, and course success rate made to the 1st year students (n:38) that took the Anatomy I course in Aydın Adnan Menderes University Veterinary Faculty. The students participating in the study were randomly divided into 3 groups. In practice lessons, Group 1 studied with the demountable skeleton model and bones; group 2 studied with the traditional (fixed at the joints) skeleton and bones, group 3 studied only with bones. In the quiz exam, 16 questions were asked to the groups about the bones of the forelimb; It was seen that the highest average of correct answers given to the questions belonged to group 1. According to the midterm exam results, it was seen that the highest-grade point average belonged to Group 1, and similarly, the success rate of the Anatomy 1-semester exam was found to be high in Group 1. In the results of the questionnaire evaluating the effectiveness of the educational materials given in the study, it was determined that the students in all groups mostly preferred to work with the demountable skeletons. According to the results of the study, it can be said that the success and motivation of the students working with the demountable skeleton are positively affected.

Keywords: *Anatomy, student, veterinarian, osteology, demountable skeleton*

Osteoloji Eğitiminde Demonte Olabilen Köpek İskelet Modelinin Kullanımı

ÖZET

Anatomi dersinin teorik kısmında verilen osteoloji bilgilerinin uygulama derslerinde daha anlaşılabilir olmasını sağlayarak; osteoloji eğitiminin etkinliğini arttırmaktır. Hayvan materyali olarak 3 adet köpek kadavrası kullanıldı. Maserasyon yöntemiyle kadavraların kemikleri temizlendi. Kemiklerin eklem yüzeyine matkapla delikler açılarak güçlü neodyum mıknatısları karşılıklı gelecek şekilde gömüldü. Mıknatıslar; kafa, mandibula, C1-C6 omurları, ön ve arka bacak eklemlerine yerleştirildi; böylece iskeletin demonte edilebilmesi sağlandı. Bu iskeletlerin osteoloji öğrenimine olası katkıları Aydın Adnan Menderes Üniversitesi Veteriner Fakültesi 2021-2022 eğitim-öğretim yılında Anatomi I dersi alan 1. sınıf öğrencilerine (n:38) yapılan anket, quiz, ara sınav, yılsonu sınavı ve ders başarı oranları göz önüne alınarak değerlendirildi. Çalışmaya katılan öğrenciler rastgele seçimle 3 gruba ayrıldılar. Uygulama derslerinde, 1. grup demonte iskelet modeli ve kemikler; 2. grup geleneksel (eklemlerinden sabitlenmiş) iskelet modeli ve kemikler 3. grup ise sadece kemiklerle çalıştılar. Yapılan quiz sınavında gruplara ön bacak kemikleriyle ilgili 16 adet soru yöneltildi; sorulara verilen doğru cevap ortalaması en yüksek grup 1'e ait olduğu görüldü. Ara sınav sonuçlarına göre en yüksek not ortalamasının Grup 1'e ait olduğu benzer şekilde Anatomi 1 dersi yarıyıl sınavı başarı oranının da Grup 1'de yüksek olduğu görüldü. Çalışmada verilen eğitim materyallerinin etkinliğini değerlendiren anket sonuçlarında tüm gruplardaki öğrencilerin çoğunlukla demonte olabilen mıknatıslı iskeletlerle çalışmayı tercih ettikleri yönünde görüş bildirdikleri tespit edildi. Çalışma sonuçlarına göre demonte olabilen mıknatıslı iskeletlerle çalışan öğrencilerin ders başarı ve motivasyonlarının olumlu yönde etkilendiğini söylenebilir.

Anahtar kelimeler: *Anatomi, öğrenci, veteriner hekim, osteoloji, demonte iskelet*

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Received Date: 29.06.2022 – Accepted Date: 14.09.2022
DOI: 10.53913/aduveterinary.1137573

Introduction

The science of anatomy has been one of the cornerstones of both veterinary medicine and human medicine education throughout history (Gültiken, 2011; Atay et al., 2016). Detailed knowledge of anatomical systems is vital for clinical veterinary practice. However, it has been reported that students have difficulties in transferring the skills they learned theoretically from textbooks to real-life applications (Canrigh et al., 2022). For this reason, the necessity of practical courses is emphasized as a basic element in the education programs of the Faculty of Veterinary Medicine. Difficulty and abstractness of the subjects that students see in theoretical lessons; also increase their need for practical lessons during their education (Altuğ et al., 2018).

Traditional anatomy education is carried out with theoretical lecture notes and practical lessons on cadavers. Today, the variety of course materials is greater. Didactic lessons, books, and atlases, dissection and cadaver applications, digital atlases, and 3D models can be counted among these (Altuğ et al., 2018). In anatomy; since the skeletal system (osteology) is the basis for other systems of the body, it is an important subject. For example; Many anatomical structures belonging to the muscle, vessel, and nervous system are named according to the bone sections where they are located, adjacent to, and passed through. Materials used in osteology education; natural bones of animal species, skeletons obtained by joining these bones with adhesives (traditional skeleton), and models made of plastic materials. It is not possible to show the relations and contacts of the bones at the joint level in traditional skeletons, which were reported to be instructive by the students. In addition, joint surfaces and anatomical structures on these faces cannot be seen in the traditional skeletons. In the case of examining the bones separately without creating a skeleton; students may have difficulty in learning about the placement order and position of the bones and have difficulty in visualizing a whole skeleton in their minds. The first aim of the study is to create skeletons that can be demountable (made of bones that can be separated from the skeleton and reassembled to the skeleton when desired) by processing the bones obtained from dog cadavers. The second aim is to investigate the effect of demountable dog skeleton and dog skeleton prepared by the traditional method (glued fixed) and bones that are not assembled as skeletons in osteology training. The hypothesis of the study; the demounted skeleton model can facilitate the comprehension of the order of placement of the bones on the skeleton (from cranial to caudal from proximal to distal), posture positions on the skeleton, directions, and articular surfaces. Thus, it can increase students' interest in the lesson and their learning success.

Materials and Medhods

The method in the study was carried out in two stages. The first stage is the preparation of the skeletons; The second stage is the use of skeletons in practice lessons, the evaluation of quizzes, surveys, midterm, and end-of-year exam results.

Preparation of skeletons

The animal material of the study was obtained from dog cadavers used for training purposes in the dissection laboratory of ADU Faculty of Veterinary Medicine, Department of Anatomy. The processes of removing the skin, muscles, and organs of the cadavers, cleaning the bones, and making them ready for the skeleton were carried out in the laboratories of the Department of Anatomy. In the study, 2 demountable (consisting of bones/joints that can be separated from the skeleton and reassembled to the skeleton when desired) and 1 traditional (all bones are fixed by gluing from the joints) dog skeleton were prepared. The skin, muscles, and organs of the dog cadavers were removed with surgical instruments such as scalpels, forceps, and curettes. The cleaning of fat, fascia, and tendon tissues on the bones was carried out by boiling. Tap water containing sodium hydroxide was used as the boiling solution. Boiling was done intermittently for 24-36 hours. The bones, which were free of tissues, were left to cool. For the complete removal of fat and tissue residues on the bones, whitening, and disinfection of the bones 50% hydrogen peroxide was used. Bones were kept in hydrogen peroxide for 20-30 minutes for this procedure. The bones were then kept in tap water for 1 day, washed, and allowed to dry. After drying, holes of 3-8 mm in diameter and 3-6 mm in depth were drilled in the joint areas of the bones with the help of a drill. Strong neodymium magnets were attached to the drilled holes with the help of the fast adhesive. The magnets were placed at the joints of the head, mandible, C1, C2, C3, C4, C5, C6 vertebrae, scapula, humerus, antebrachium, coxae, femur, tibia, patella, fabella, carpal, and tarsal bones as well as some of the roots of the canine and molar teeth (Figures 1-2-3). Thus, the bones were ensured that they could be separated from the joints between the mentioned bones when desired. The vertebrae were kept in their natural position using a supporting iron but they could be demountable if desired, with the aid of magnets. The scapula was attached to the body with the help of magnets. The whole skeleton was obtained in the natural standing position with the bones that could be demountable from the joint areas by means of magnets and support materials. The skeletons were made ready by placing them on the platforms (Figure 4). For the traditional skeleton construction, all operations except the placement of magnets were carried out sequentially. The bones were fixed with instant glue so that they would not be separated from the articular surfaces.

Use of skeletons in practice lesson

Aydın Adnan Menderes University Faculty of Veterinary Medicine Anatomy I course is given to the first-year students with a systematic method in the first semester. Students take general anatomical terminology and general osteology in the first week. Then they complete the forelimb osteology course in two weeks. The success and satisfaction of the students participating in the study in the forelimb osteology course were evaluated. In order

Figure 1. Placement of magnets (black arrow) on the cervical vetrebrae

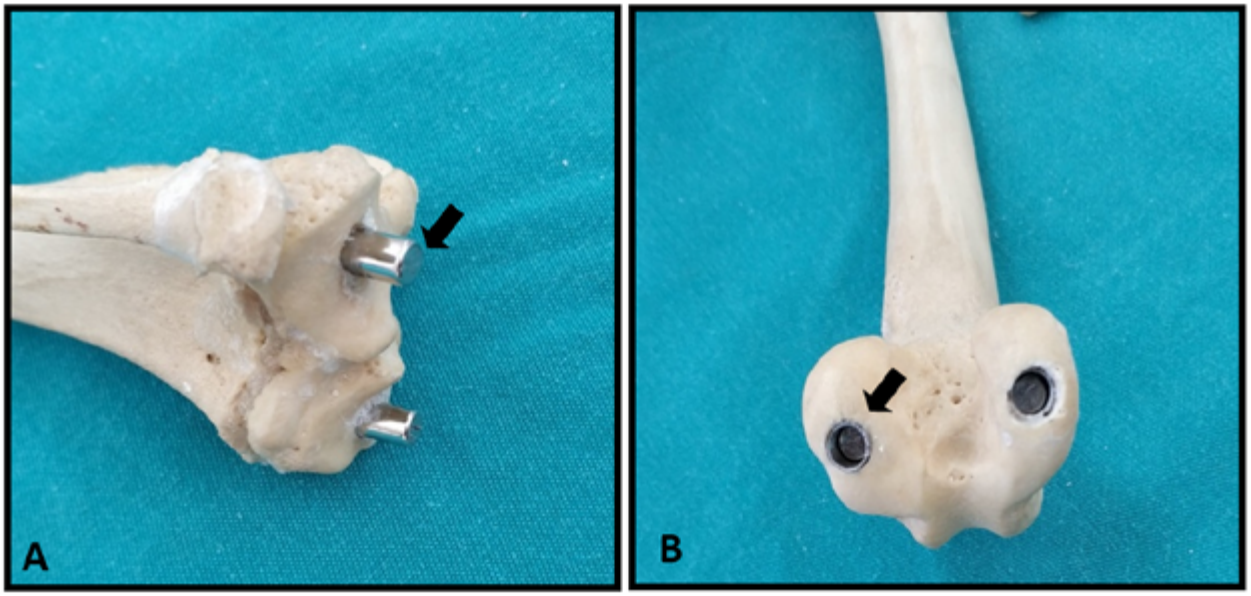

Figure 2. Placement of magnets (black arrow) on the articular surfaces of the tibia (A) and femur (B)

to evaluate the possible contributions of demountable skeletons to the osteology course, the students who took the anatomy I course were provided to use the skeletons in the practice courses (Figure 5). For this purpose, students (n:38) were randomly divided into 3 groups. The students were informed about the study and the groups; It was announced that any personal (age, gender, name, surname) data of the students participating in the groups would not be used in the study and their consent was obtained. In the osteology course, students work with the bones of the related topics. However, the students participating in the study were given a demountable skeleton or a traditional skeleton beside the bones for the practical lesson. Thus, 3 groups were formed (Table 1).

The subjects of forelimb osteology were taught to the students in all groups by the same lecturer from the Department of Anatomy in the practice course. After the forelimb osteology course was completed, a quiz exam consisting of 16 questions was given to the groups.

Students took the exam one by one; questions were directed to the students orally; each correct answer is marked as +1. The quiz was administered by the faculty member of the anatomy department, who explained the forelimb osteology to the student groups. Afterward, it was ensured that the demountable skeleton and traditional skeleton were used alternately between all groups during 2 lesson hours. A questionnaire (Appendix: 2) was conducted to the students participating in the study on whether demountable skeleton, traditional skeleton, and bones could be preferred as course materials. The success grades of the groups in the midterm exam and the correct answer rates given to the forelimb osteology subjects in the midterm exam were evaluated. At the end of the semester, the students' end-of-year exam success was also evaluated.

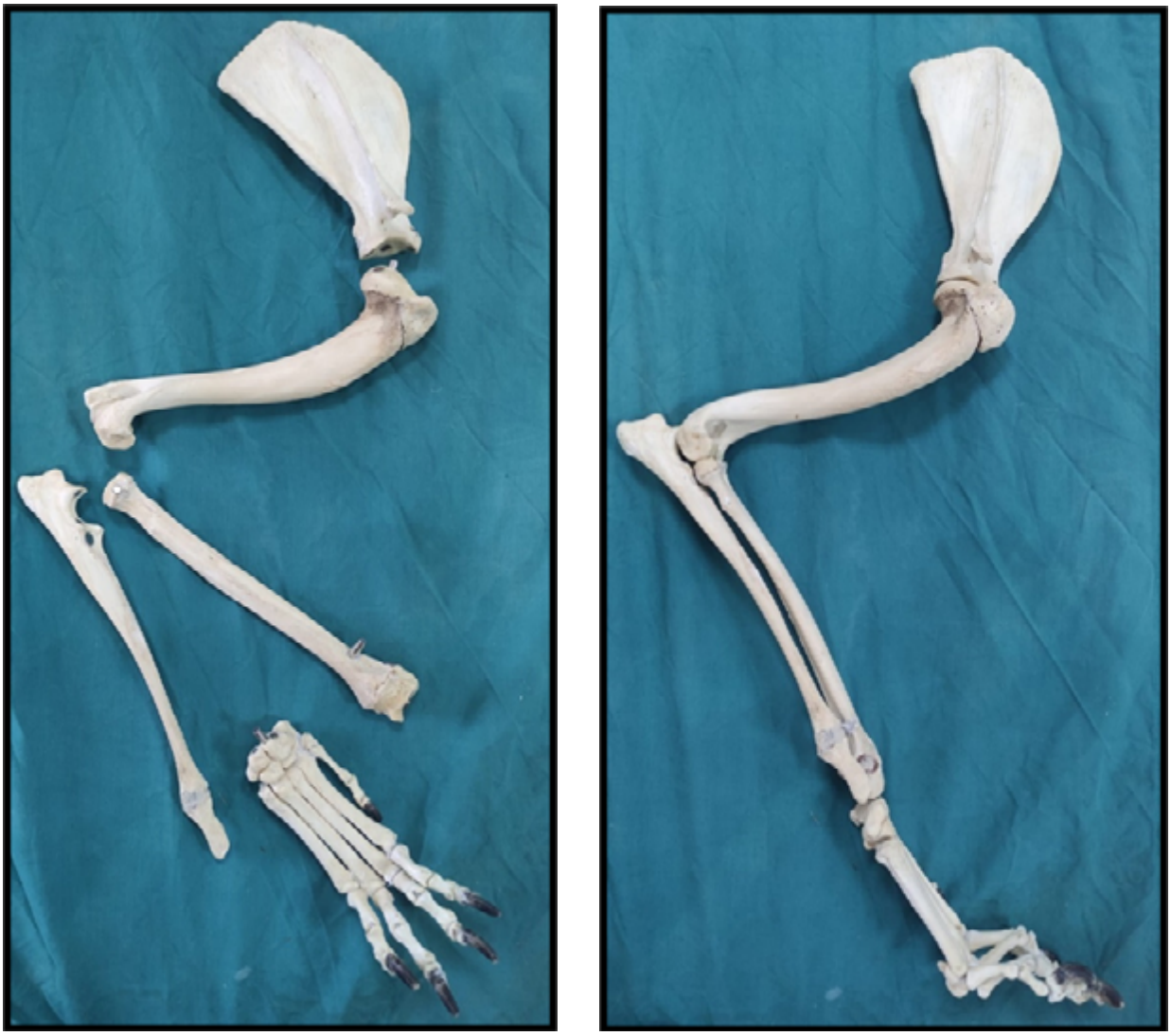

Figure 3. Demounted front leg bones (A), bringing the forelimb bones together with the help of magnets (B)

Figure 4. General view of demountable fore and hind limb bones, separated

Figure 5. Students studying with demountable skeleton

Ethics committee approval

Live animals were not used in our study. According to Article 8/19-k of the regulation on the working procedures and principles of animal experiments ethics committees, studies on "dead animal or its tissue, slaughterhouse materials" are not subject to permission. In addition, ethics committee approval was not obtained because the questionnaire and quiz exam applied to the students consisted of questions that did not contain personal information and only received opinions and answers for the evaluation of the educational materials.

Statistical analysis

The SPSS© 22.0 (Statistical Package for the Social Sciences 22) program was used for statistical analysis. The conformity of the students' grade point averages to the normal distribution was examined using the Shapiro-Wilk test. In terms of midterm, final, and make-up grades, a one-way analysis of variance (ANOVA) was used to determine whether there was a statistical difference between the groups. Scheffe post hoc test was used to determine the group or groups with a significant difference as a result of the analysis. For statistical significance, the type-I error level was determined as P<0.05 (Özdamar, 2015). The answers given by the student groups for each question in the quiz exam are correct and incorrect; At the end of the semester, the success of the anatomy course was categorized as passing and failing, and whether there was a statistical difference between the expected and observed levels of these categorical variables within the groups was determined using the "Pearson chi-square" method. Cases with a P-value below 0.05 were considered statistically significant (Hayran and Hayran, 2011). The reliability of the answers given to the questionnaire (Table 2) items was determined by the Cronbach's alpha coefficient (α). Cronbach's alpha coefficient 0-0.49 unreliable, 0.50-0.59 bad, 0.60-0.69 suspicious, 0.70-0.79 acceptable, 0.80-0.89 good, 0.90 values between -1.00 were considered as perfectly reliable (Alpar, 2016).

Results

In the study, the quiz, questionnaire, and midterm exam results of group 1 (demountable skeleton and bones), group 2 (traditional skeleton and bones), and group 3 (bones only) and the correct answer given to the questions about the forelimb bones in the midterm exam results were evaluated. rates, final exam, make-up exam results, and anatomy I course success (pass-fail) results were evaluated.

Evaluation of quiz results

The quiz consisting of 16 questions was evaluated over a total of 16 points, each correct answer being +1 point. A statistically significant difference was found between the total correct answer mean of group 1 and group 2, but no statistical difference was found between the other groups (Table 3).

In the statistical evaluation of the correct and incorrect answers given to each quiz question between the groups, a significant difference was found in the answers given to the 12th, 13th, and 14th questions. These questions are about the antebrachium bones; The frequency of correct answers to these questions higher than the expected level belongs to group I.

Evaluation of the survey results

A 6-item Likert-type questionnaire and 1 open-ended question were administered to the groups. The Cronbach's alpha coefficient (α) was 0.664. Survey work; A total of 34 people, including 12 students from group 1, 12 students from group 2, and 10 students from group 3 (Figure 6).

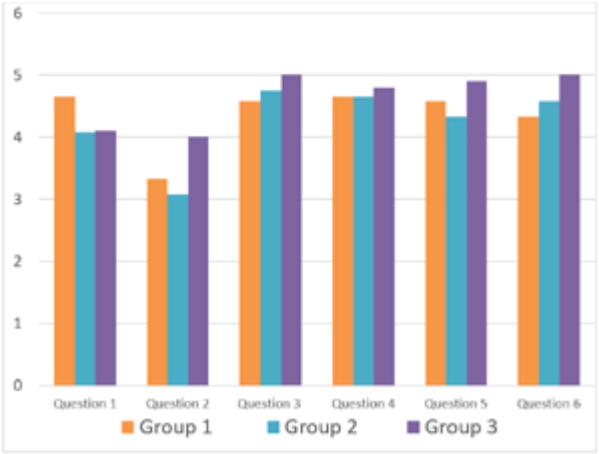

Figure 6. Questionnaire result

Anatomy lesson; Statistical evaluation of midterm, final, and make-up exams:

After the evaluation of the quiz and survey results of the groups, the exam success of the students was also exam ined in order to evaluate the general course success status of the students.

As a result of the statistical evaluations, based on midterm, final, and make-up grades, among group 1, group 2, and group 3; no difference was found for midterm and make-up exams; A statistically significant result was found between group 1, group 2 and group 3 for the end-of-year exam (Table 4).

Table 1. Students groups

Group	The course material used
1 (n:14):	Students working with demountable skeletons and bones
2 (n:12):	Students working with traditional skeletons and bones
3 (n:12):	Students working only with bones

Table 2. Questionnaire

1.	It is instructive to work with bones (not skeletally combined) in the skeletal anatomy course.
2.	It is instructive to work with skeletons with all bones joined in the skeletal system anatomy course.
3.	In the skeletal system anatomy course, it is instructive to work with skeletons whose bones can be demountable.
4.	Demountable skeletons allow easier grasping of the stance positions of the bones on the skeleton.
5.	Demountable skeletons provide a better understanding of the anatomical structures of the area where the bones articulate.
6.	The demountable skeleton model has advantages over other materials used in osteology education, such as increasing the interest in the lesson and making the lesson more productive and enjoyable.

The numbers from 1 to 5 correspond to the following expressions.
1: No, 2: partially, 3: could be, 4: correct, 5: absolutely right

Table 3. Quiz result, SD: standard deviation

	Group 1 (n=14) mean ± SD	Group 2 (n=14) mean ± SD	Group 3 (n=14) mean ± SD	P
Total correct answer average	11.78±0.80[a]	8.08±0.97[b]	9.16±0.91[ab]	0.015

[a, b]: The difference between the groups with different letters in the same line is statistically significant (P= 0.05)

Table 4. Anatomy lesson; Evaluation of midterm, end-of-year and make-up exam results, SD: standard deviation

	n	Group1 mean ± SD	n	Group2 mean ± SD	n	Group 3 mean ± SD	P
Mid-term exam	14	67.29±3.90	12	54.25±5.40	12	58.25±4.87	0.138
End of year exam	14	64.64±4.69[a]	12	45.58±5.34[b]	12	36.66±4.86[b]	0.001
Makeup exam	4	54.00±3.71	6	38.83±8.32	4	40.75±11.79	0.456

[a, b]: The difference between the groups with different letters in the same line is statistically significant (P= 0.05)

Table 5. Evaluation of Anatomy I course pass-fail rate

		Exam result		Total
		Pass	Fail	
Group 1	n	10	4	14
	%	71.4%	28.6%	100.0%
Group 2	n	7	5	12
	%	58.3%	41.7%	100.0%
Group 3	n	4	8	12
	%	33.3%	66.7%	100.0%

Statistical evaluation of Anatomy I course pass-fail rate

As a result of the chi-square analysis, it was determined that there was no statistically significant difference at the expected level in terms of the number of passing failures for each group of students (X^2: 3.860 P=0.145). In the study, the rate of the passing of the group working with the demounted model (1st group) was higher than the other groups, but no statistically significant difference was found (Table 5).

Discussion

Anatomy is a branch of science in which visual materials, which have an important place in the education programs in medicine, are frequently used. It is stated that visual education materials and methods are more effective in teaching and learning processes than auditory methods and other methods. This situation reveals the importance of practice lessons and course materials in anatomy learning (Özdemir, 2003). Within the scope of the current study, a demountable dog skeleton, which is an application course material, was created. With the use of this material, it is aimed to make the forelimb osteology subjects given in the theoretical anatomy lesson more understandable in the practical lessons and to increase the efficiency of the osteology education.

When scientific studies in the field of anatomy were evaluated in our country, it was reported that the most studies were on the locomotor system with a rate of 20.1%, and the animal species used in these studies were dogs with 12.35% (Dilek et al., 2019). Today, it is seen that the animal species brought to veterinary clinics with the highest rate is the dog (İşler et al., 2015; Şimşek and Akçay, 2021). For this reason, especially dogs were preferred as animal material in the study. In addition, the placement of the magnets used in the study of the dog skeleton and the convenience of keeping the skeleton in disassembled-assembled form was also effective in the selection of animal material.

It was predicted that the practice course with demountable dog skeleton, which was carried out within the scope of this research, could be more instructive than the traditional skeleton and bones. No other study investigating the results of the practical training given with the demountable skeleton was found in the literature. Therefore, we could not reach the articles where we can discuss the results of our study. After the forelimb osteology topics were given to the student groups in the study (1.2.3. groups), a quiz exam consisting of 16 questions was applied. Quiz exam questions are prepared to determine the cranial-caudal, proximal-distal placement orders of bones on the skeleton, their posture positions, and directions (cranial-caudal, lateral-medial) on the skeleton. In the quiz results, a statistically significant difference was found between the total correct answer mean of group 1 and group 2; no statistically significant difference was found between the other groups. The highest correct answer average given to the quiz questions consisting of a total of 16 questions belonged to the 1st group (11.78). Antebrachium, which is one of the forelimb bones, consists of two bones, the radius, and the ulna. These bones join the synovial joint at two proximal and distal points in the dog (Öcal et al., 1998). The orientation and placement of these two bones in dogs may be more difficult for students to comprehend than other forelimb bones. The 12th and 13th questions of the quiz are about the aspects of the antebrachium bones and their placement on the skeleton. The 14th question indicates the *incisura radialis ulnae*, which is the synovial articular surface located proximal to the ulna. When the answers given to the 12th, 13th, and 14th questions between the groups were evaluated, it was seen that the group 1 students had a higher correct answer frequency than the expected level. In the quiz results, it was determined that the students working with the demountable skeleton model (1st group) were more successful than the other groups; It has been observed that the bones have a better grasp of their position and orientation on the skeleton. There are studies investigating the success and satisfaction levels of anatomy courses in both human medicine (Arı et al., 2003; Sindel et al., 2008; Atay et al., 2016; Yavuz et al., 2017) and veterinary medicine (Gültiken, 2011; Özen et al., 2012; Altuğ et al., 2018). In the current study, the demountable skeleton and the traditional skeleton were used alternately between all groups, and which educational material the students preferred in terms of osteology education was investigated with a questionnaire. The reliability of the answers given to the questionnaire statements was determined by Cronbach's alpha coefficient (α). In the study, it was observed that this coefficient was 0.664, so the questionnaire was found suspicious.

The main reason for calculating the reliability coefficient as suspicious is thought to be the low number of students participating in the survey. The number of students included in the study is one of the limitations of the current study, and it can be suggested that similar studies be carried out with larger student groups. When the results of the 6-item Likert-type are evaluated, it is seen that the 3rd, 4th, 5th, and 6th propositions regarding the use of the demountable skeleton model were approved with the highest score. The students in the 2nd and 3rd groups who did not use the demountable skeleton model during their studies were the students who gave the highest scores to these propositions. The common opinion of all groups in the answers given to the open-ended question of the questionnaire; "Because the bones are removable in the demountable skeleton model, it is more preferable in terms of understanding their postures and directions". According to the results of the current study, it can be said that the demountable skeleton model is preferable to other materials as an osteology course training material. When the course-exam success of the groups is evaluated; between Group 1, Group 2, and Group 3; there was no statistical difference for midterm and make-up exams; a statistically significant result was found between Group 1, Group 2, and Group 3 for the end-of-year exam. The highest-grade point average obtained in these exams belongs to the

1st group. When only the correct answers to the forelimb osteology questions were evaluated in the midterm exam, it was seen that the highest correct answer rate belonged to the 1st group. When Anatomy I course pass-fail rates are evaluated, there is no statistically significant difference between the groups; It is seen that group 1 has the highest passing rate with 71.4%. It is thought that there is not enough data to say that the results of both the end-of-year and make-up exams, and therefore the passing rates, are completely dependent on this study. However, when the quiz, survey, and exam success results of this study are evaluated, it can be said that the learning of the 1st group, who uses the demountable skeleton model, has a positive effect on their learning. The main reasons that led to this success; as stated in the survey results, the demountable model may increase the interest in the lesson and maybe the motivation for the student to succeed in the Anatomy I lesson after the high midterm exam grade (1st group). It can be thought that many anatomical structures in the muscle, vascular and nervous systems, which are taught following the skeletal system anatomy course, are named after the bone sections where they are located, adjacent to, and pass through, leading to ease of learning and increasing motivation in students.

Conclusion

When the results of the current study are evaluated collectively; the demountable skeleton model facilitates the comprehension of the placement of bones on the skeleton, posture positions, directions, and joint surfaces; It is seen that the hypothesis that it can positively affect students' interest in the course and thus learning success has been largely confirmed. However, it will contribute to obtaining clearer results if the study is carried out with a demountable skeletal model in a large-participation student group to cover all osteology topics.

Acknowledgements

This study was supported by TÜBİTAK 2209-A program. Application number:1919B012003669. The authors thank TÜBİTAK for their support.

Conflict of Interest

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

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Animal Health Prod and Hyg (2022) 11 (2) : 9 - 14

Animal Health, Production and Hygiene

www.dergipark.or.tr/tr/pub/aduveterinary

Research Article

Investigation of Nitrate, Nitrite Pollution Levels and Organic Matter Amounts of Artesian Waters of Aydın Region

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ABSTRACT

Among the pollutants that can spread over large areas, nitrate, nitrite and organic matter are considered as an important indicator of pollution of the environment. It is known that there is a high connection and relationship between agricultural activities and nitrate contaminations. This study was carried out to determine the amount of nitrate, nitrite and organic matter, which are important indicators of water pollution, in artesian waters taken from various districts of Aydın. The artesian waters used in the study were obtained from randomly selected artesian wells close to the settlements of 9 districts of Aydın province (Germencik, Çine, Kuşadası, Bozdoğan, Efeler, Köşk, Söke, Yenipazar, Koçarlı) in September 2019. Nitrate and nitrite analysis in waters by colorimetric method; organic matter analysis was performed by titrimetric method. Underground water resources in Aydın are generally used for agricultural irrigation and to meet the water needs of farm animals. In the water samples taken in the study, nitrate and nitrite amounts were determined below the upper limit determined by TSE. Despite this, the amounts of organic matter were found in all samples; It is above the limit values of 0.1-2 mg/L for groundwater. It is thought that this contamination due to organic matter may have been caused by domestic contamination.

Keywords: *Aydın, nitrate, nitrite, organic matter, water analysis*

Aydın Bölgesi Artezyen Sularının Nitrat, Nitrit Kirlenme Düzeyleri ve Organik Madde Miktarlarının Araştırılması

ÖZET

Geniş alanlara yayılabilen kirleticiler arasında nitrat, nitrit ve organik madde çevrenin kirletilmesinde önemli bir gösterge olarak kabul edilmektedir. Tarımsal faaliyetler ve nitrat kirlenmeleri arasında yüksek bir bağlantı ve ilişki olduğu bilinmektedir. Bu çalışma, Aydın iline bağlı çeşitli ilçelerden alınan artezyen sularında su kirliliğinin önemli belirteçlerinden olan nitrat, nitrit ve organik madde miktarlarını belirlenmek amacıyla yapılmıştır. Çalışmada kullanılan artezyen suları, 2019 yılı Eylül ayında Aydın ilinin toplam 9 ilçesinden (Germencik, Çine, Kuşadası, Bozdoğan, Efeler, Köşk, Söke, Yeni Pazar, Koçarlı) yerleşim yerlerine yakın rastgele seçilmiş artezyen kuyularından temin edilmiştir. Sularda nitrat ve nitrit analizleri kolorimetrik metotla; organik madde analizi titrimetrik yöntemle yapılmıştır. Aydın'daki yeraltı su kaynakları genellikle tarımsal sulama ve çiftlik hayvanlarının su ihtiyaçlarını gidermek için kullanılmaktadır. Yapılan çalışmada alınan su örneklerinde nitrat ve nitrit miktarları TSE'nin belirlediği üst limitin altında tespit edilmiştir. Buna rağmen organik madde miktarları tüm örneklerde; yeraltı suları için belirlenen 0,1-2 mg/L limit değerlerin üzerindedir. Organik maddeye bağlı bu kirlenmenin evsel kirlenmeden kaynaklanmış olabileceği düşünülmektedir.

Anahtar kelimeler: *Aydın, nitrat, nitrit, organik madde, su analizi*

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Received Date: 07.07.2022 – Accepted Date: 03.10.2022

DOI: 10.53913/aduveterinary.1141926

Introduction

Water is an indispensable substance that plays the most important biological and chemical role in the universe. It is of vital importance for all known living forms and for us humans. In addition to these, it is one of the cornerstones of the life cycle not only for living things and people, but also socially and eco-systemically. If the oceans, seas and polar glaciers remain on one side, only 3.5% of the existing water in the world is usable (Kılıç, 2008).

A large part of the water used in daily life is obtained from underground sources. In recent years, pollution from industrial and domestic wastewater and the use of numerous chemical fertilizers in agricultural systems have caused pollution in groundwater. Pollution is an important issue in environmental sustainability and a serious environmental problem for the world (Zhang et al., 2018).

The excessive use of nitrogen fertilizers in agriculture has been one of the primary sources of high nitrate in groundwater. Nitrogen is applied in the forms of ammonium (NH_4+) and amide (NH_2-), which are converted to nitrate in the soil system through very rapid mineralization in tropical and subtropical soils. Due to its high solubility in water and low retention by soil particles, nitrate is prone to leaching into subsoil layers and eventually groundwater if not taken up by plants or denitrified to N_2O and N_2 (Majumdar and Gupta, 2000; Burkut, 2018). The rate of infiltration is governed by the soil properties and the amount of water present in the soil system. The use of nitrogen fertilizers, excessive application of irrigation and abundant precipitation can increase the transfer of nitrate to groundwater. Animal farms, barns, septic tanks, animal and human contamination are other important sources of high nitrate contribution to groundwater. Nitrite ion is formed as a result of nitrate oxidation or more nitrate reduction during chemical and biological processes (Mikayilov and Acar, 1998; Atay and Pulatsu, 2000; Şanlı, 2002; Atılgan et al., 2007).

The importance of nitrates and nitrites in terms of environmental toxicology is evaluated in relation to acute and chronic nitrate and nitrite poisoning caused by dose intake in humans and animals. Nitrates, which are taken into the body through food and water, are reduced to hydroxylamine and ammonia, partly by microorganisms in the intestinal flora and partly by some other factors (Lundberg et al., 2018). In the acid medium, this reduction product of nitrate reacts with amines and amides to form N-nitroso compounds. These compounds are known to have carcinogenic effects in humans and animals (Tricker and Preussman, 1991; Weyeret al., 2001; Çakmak et al., 2009). To protect public health from hazards associated with high nitrate concentration, WHO determined that nitrate is 50 mg/L and nitrite, 3 mg/L in drinking water (WHO, 2017).

High nitrate concentration in drinking water increases the risk of disease and health effects such as methemoglobinemia, diabetes, spontaneous abortion, thyroid disease and stomach cancer (Havelaar and Melse, 2003; Kobayashi, 2018; Ward et al., 2018; Carlström et al., 2020). Depending on the amount of nitrate taken with water, acute and chronic poisoning is seen in animals. Water containing more than 500 ppm nitrate can cause anoxic convulsions, cyanosis of the mucous membranes and non-pigmented parts of the skin, unconsciousness, coma and acute poisoning causing death; long-term consumption of nitrate waters higher than 125 ppm causes respiratory distress, vasodilation, tachycardia, mydriasis, cyanosis, decreased milk secretion, abortion, avitaminosis, deterioration in thyroid gland functions, and decreased thyroxine, triiodotronin and somatomedin levels (Kaya et al., 1998; Ateşşahin and Servi, 2017).

Therefore, the identification of nitrate sources is crucial for planning effective nitrogen pollution reduction strategies and ensuring the sustainability of water resources. Understanding the nitrate contamination situation and determining the source of nitrate pollution is key to pollution control.

One of the most important parameters for understanding the quality of water is the amount of organic matter. If the amount of organic material in the water is too large, serious results may occur. Organic substances found in natural waters are composed of naturally occurring organic substances in the aquatic environment; it arises from the substances that come out during the treatment, disinfection and distribution of water, or from the pollutants that arise as a result of the pollution of water resources by industrial and agricultural activities. Pollutants mixed with industrial and domestic wastewater and agricultural fields are important polluting sources that affect water quality. An increase in the amount of organic matter is a sign of water pollution. Organic substances cause bacteria and fungi to grow in water (Alaş and Çil, 2002). It is undesirable for the amount of organic matter in drinking water to be over 3.5 mg/L (Varol et al., 2008).

This study was carried out to determine the amount of nitrate, nitrite and organic matter, which are important indicators of water pollution, in artesian waters taken from various districts of Aydın.

Materials and Methods

The artesian waters, which are the study material, were collected from 9 districts of Aydın province (Germencik, Çine, Kuşadası, Bozdoğan, Efeler, Köşk, Söke, Yenipazar, Koçarlı) randomly selected from artesian wells close to settlements in September-October 2019, with aluminum foil taken in coated plastic bottles as 1 liter. Analyzes were performed immediately without waiting for samples.

Determination of nitrite in water samples using a nitrite reagent containing tartaric acid, α-naphthylamine and sulfanilic acid at a wavelength of 543 nm was done spectrophotometrically. The nitrite amounts were determined from the calibration curve created with 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 and 1 mg nitrite standard solutions prepared with $NaNO_2$ (Anonymous, 2013).

Brucine method is one of the most used methods in the chemical determination of nitrate ion in water. The basis of the method is to read the absorbance values of the yellow color formed in the reaction between brucine and nitrate in the spectrophotometer and to compare these values with standard solutions. 2 ml of water samples were taken, 1 ml of brucine sulfanilic acid solution and 10 ml of sulfuric acid were added. The prepared mixtures were kept in the dark for 10 minutes. Then, 10 ml of distilled water was added to them and mixed. After cooling for 20-30 minutes in the dark, measurements were made in the spectrophotometer at a wavelength of 410 nm. Concentrations were calculated from the calibration curve (Anonymous, 2013; Baltacı, 2000).

Organic substances can enter waters from a variety of sources, including plants, humans and animals. One of the methods used in the determination of organic matter in water is the titration method. It is based on the oxidation of organic substances in water by using permanganate solution in an acidic environment. The amount of potassium permanganate spent in oxidation by back titration is determined and the amount of organic matter is calculated from there.

25 ml samples were taken into 250 ml flasks. 2.5 ml of sulfuric acid solution and 2.5 ml of standard potassium permanganate solution were added and kept in a boiling water bath for 30 minutes. After waiting, 2.5 ml of sodium oxalate was added to it and titrated with potassium permanganate until pink color appeared in hot state, and the consumptions were noted. With the help of the formula, the amount of organic matter in the sample was calculated (Anonymous, 2013).

$$OM = \frac{S}{m} \times 1000 \times 0{,}0125 \times 8$$

OM: Organic matter amount (mg/L)

Q: Amount of $KMnO_4$ used (ml)

m: Sample amount (ml)

Results

The nitrite amounts of the samples, the absorbance values read at 543 nm, were calculated according to the graphic formula (y = 0.8438x; r^2=0.998) of the calibration curve created from the standard solution series.

Calibration curve graphic formula of standard solutions with nitrate varying between 0-50 mg/L; found as (y = 0.8438x; r^2=0.998). By replacing the read absorbance values in this formula, the concentrations of the samples were determined. The nitrate and nitrite amount of the water samples taken from the districts are presented in Table 1. The amounts of organic matter in the samples determined by the titration method are given in Table 1.

The highest nitrite amount is in the water sample taken

Table 1. Nitrite, nitrate and organic matter results of water samples

Districts	Nitrite Amount (mg/L)	Nitrate Amount (mg/L)	Organic Matter Amount (mg/L)
Germencik 1	0.012	0.069	6.4
Germencik 2	0.003	0.216	6
Germencik 3	0.0008	0.40143	6
Germencik 4	0.0034	2.417	6
Germencik 5	0.0042	0.2239	5.8
Germencik 6	0.0802	1.5748	5.8
Efeler	0.0118	0.06176	6
Kuşadası	0.0017	2.1075	6
Bozdoğan	0.0017	0.1698	6.4
Söke 1	0.016	0	6
Söke 2	0.0178	0.03088	7.8
Köşk	0.0135	1.7215	6
Koçarlı	0.027	0	6
Işıklı(Efeler)	0.0135	0.04632	6.2
Umurlu(Efeler)	0.0397	1.25061	6
Yenipazar	0.1173	0.17756	5.4
Çine	0.3502	0	5

from Çine district, the nitrate amount was determined in the samples taken from Germencik 4 and Kuşadası. The amount of organic matter in all samples was found to be between 5-7.8 mg/L.

Discussion

The biggest cause of groundwater pollution is the discharge of domestic and industrial wastes to the receiving environments without treatment. After solid, liquid and gaseous wastes are given to the receiving environment; depending on the climatic situation, the structure of the soil, the shape of the land, the type of waste and time, it mixes with groundwater. In addition, excessive and unconscious use of agricultural struggle drugs is an important cause of pollution. Where there is no sewage system, dirty waters leaking from toilet pits and fertilizers are mixed with groundwater to pollute ground water.

Nitrate among pollutants that can be spread across large areas is considered an important indicator of pollution in the environment. Many studies on this subject show that there is a high correlation and relationship between agricultural activities and nitrate pollution (Olhan and Ataseven, 2009). According to the drinking water standard TSE 266, nitrate concentration was determined as 45 mg/L (Sağlam, 2000).

In a study conducted around Karabük Eskipazar district, nitrate pollution in discharged waters was investigated and high nitrate concentration was encountered. Nitrate values were found to be minimum 1.2 mg/L and maximum 135 mg/L and the mean value was 48 mg/L (Ekemen Keskin, 2010). In Manisa (Eryurt and Sekin, 2001), which depends on fertilization and agricultural activities; In the vicinity of Urla and Menemen (Aslan and Akkaya, 2001), high rates of nitrate contamination have been detected in groundwater.

In the study of Ekşi (2005) in Samsun province, 9 water samples were analyzed. In addition to having a very high value in well water, it has been reported that it is lower than the limit value (45 mg/L) in mains water. According to the results of the monitoring study conducted on the basis of nitrate parameter at 15 selected groundwater sampling points in Eskişehir between October 2005 and July 2006, it was observed that the nitrate concentrations ranged from 13 to 360 mg/L and the standard value (50 mg/L) was exceeded at 6 groundwater sampling points (Çakmak, 2007). The amount of 243.61 mg/L nitrate and 0.63 mg/L nitrite (Özdemir et al.,2004) was found in the Afyon region well waters. Nitrate levels in the well waters of Van center and its districts are 24.7-35.5 ppm; nitrite levels were determined below 0.1 ppm (Ağaoğlu et al., 2007). In the province of Bitlis, nitrate and nitrite amounts in natural spring waters used for human consumption were found to be below 0.5 mg/L (Urgan and Koç, 2020).

In the studies where the seasonal evaluation of nitrate pollution in groundwater in İzmir was carried out, different nitrate values were determined according to the regions; It has been observed that nitrate levels in the waters tend to decrease in winter and increase in summer (Polat et al., 2007; Tayfur et al., 2008). In a similar study, Esmeray and Gökçekli (2020) determined nitrate concentrations in 22 groundwater sources in Karabük province from 2.50 to 44.20 mg/L in the rainy season; they reported a slight increase in the dry season, ranging from < 0.45 to 50.5 mg/L.

In the artesian waters of Aydın province, 1.11–158.9 ppm in winter and 1.2–96.87 ppm in summer nitrate were detected. While 1.26 ppm nitrite was detected in artesian waters in winter only in two settlements, nitrite was not found in other places. It has been determined that the residential areas where the nitrate level of the water samples is determined to be 40 ppm and above in winter and summer months are on flat plains and are places where intensive agriculture and animal husbandry are made (Uçmaklıoğlu, 2011).

There are around 15000 wells, most of which are for irrigation purposes, that have been drilled throughout the province of Aydın. As a result of the researches carried out by the DSI Regional Directorate in 2010, ammonia, nitrite, nitrate was found in various purpose wells drilled in Aydın city center and throughout the province, and an average of 40 mg/L pollution was detected in the measurements (Arslan and Şenol, 2010). The nitrate results of underground (geothermal) waters taken from different stations in Aydın province were 0.063-42.22 mg/L; nitrite was found between 0.008 and 43.624 mg/L. The highest nitrate-nitrite amount was determined in Alangüllü town, which is rich in geothermal waters (Özdoğan et al., 2016).

In this study, which was carried out in the artesian waters of Aydın center and its districts, the average nitrate and nitrite levels were found to be lower than the average values reported by many researchers. It was observed that the nitrate level of artesian waters remained below 45 ppm, which is the permissible level determined in drinking water for humans. According to TSE 266, nitrite is not required in drinking water, while nitrite is allowed up to 0.05 ppm in drinking water according to the Regulation Concerning Water Intended for Human Consumption of the Ministry of Health (Sağlam, 2000). While the nitrite level was below 0.05 ppm in the analyzed samples, it was found to be high in the water samples taken from Yenipazar, Germencik 6 and Çine.

The determination of total organic matter is the measurement of reducing substances present in the minimum amount of water. Total organic matter can be correlated with the natural color of the water or with some organic waste from industrial wastes or sewers. An increase in the amount of organic matter indicates that there is pollution in the water. Atasoylu et al. (2006) were determined the amount of organic matter above 3.5 mg/L in 154 of 4410 drinking water samples by Aydın provincial public health laboratory. The nitrate and nitrite amount in the water samples taken in the study were determined below the upper limit determined by TSE. Despite this, the amount of organic matter in all samples; It is above the 0.1-2 mg/L limit values determined for groundwater.

It is thought that this pollution due to organic matter may have resulted from domestic pollution.

Conclusion

Groundwater management is key and ensuring the sustainable use of groundwater resources is a major challenge. For this reason, water analysis in terms of water pollution should be repeated from time to time, taking into account seasonal changes.

Acknowledgment

This study was supported by Aydın Adnan Menderes University Scientific Research Projects as Project No: VTF 18043.

Conflict of Interest

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

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Animal Health Prod and Hyg (2022) 11 (2) : 15 - 20

Animal Health, Production and Hygiene

www.dergipark.or.tr/tr/pub/aduveterinary

Research Article

Effect of Tibial Plateau Leveling Osteotomy on Mechanical Tibial Axis Shift in Dogs: Two-Dimensional Bone Study in Sagittal Plane

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ABSTRACT

To determine the tibial axis shift (TAS), change in tibial plateau angle (TPA), and the position of the tibial articular surface after tibial plateau leveling osteotomy (TPLO) simulation in the tibiae of dogs, tibias of 91 dogs from 23 different breeds were used. The TPA, tibial length, midshaft width, medial tibial condyle length, and distances between the tibial axis (TA) and cranial-most point of the medial condyle were measured on the tibial images. After simulated TPLO, all measurements were retaken. Paired t-tests were used to compare pre- and postoperative variables. Pearson correlation analysis was conducted to assess whether the difference between pre- and postoperative TAS was related to tibial length, tibial width, medial condyle length, and/or the degree of TPA rotation. The results showed that TPLO shifted the cranial-most point of the medial condyle caudally and distally, and the caudal-most point caudally and proximally. These shifts resulted in significant differences between pre- and postoperative distances between the TA and the cranial-most point and its percentage to the medial condyle length. It is concluded that the TPLO led to caudal TAS and altered the position of the articular surface of the medial condyle with respect to the distal part of the tibia.

***Keywords**: Dog, tibia, tibial axis, Tibial Plateau Leveling Osteotomy*

Köpeklerde TPLO'nun Mekanik Tibial Eksen Kayması Üzerine Etkisi: Sagital Düzlemde İki Boyutlu Kemik Çalışması

ÖZET

Köpeklerde tibial plato düzleştirme osteotomisi (TPLO) simülasyonu sonrasında tibial eksen kaymasını (TAS), tibial plato açısındaki (TPA) değişimi ve tibial eklem yüzeyinin konumunu belirlemek için, 23 farklı ırktan 91 köpeğe ait tibialar kullanıldı. TPA, tibial uzunluk, "midshaft" genişliği, tibianın condylus medialis uzunluğu ve tibial eksenle (TA) condylus medialis'in en cranial noktası arasındaki mesafe tibia görüntüleri üzerinden ölçüldü. Simülasyonu gerçekleştirilmiş TPLO'dan sonra tüm ölçümler tekrar gerçekleştirildi. Pre- ve postoperatif değerleri karşılaştırmak için Paired t-test kullanıldı. Pre- ve postoperative TAS değerleri ile tibianın uzunluğu, tibianın genişliği, condylus medialis uzunluğu, ve/veya TPA'nın rotasyon derecesi arasında ilişkinin farklı olup olmadığı değerlendirmek için Pearson korelasyon analizi yapıldı. Çalışmanın sonuçları, TPLO ile medial condylusun en cranial noktasının caudale ve distale, en caudal noktasının ise caudale ve proximale doğru kaydığını gösterdi. Bu kaymalar, operasyon öncesi ve sonrası TA ile en cranial nokta arasındaki mesafe değerinde ve en cranial noktanın condylus medialis uzunluğundaki yüzde değerinde önemli farklılığa neden oldu. TPLO'nun, caudal TAS ve medial condylusun eklem yüzeyinin, tibianın distal parçasına göre pozisyonunu değiştirdiği sonucuna varıldı.

***Anahtar Kelimeler**: Köpek, tibia, tibial eksen, Tibial Plato Düzleştirme Osteotomisi*

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Received Date: 28.07.2022 – Accepted Date: 26.10.2022
DOI: 10.53913/aduveterinary.1150162

Introduction

A large tibial plateau angle (TPA) may lead to an increase in cranial tibiofemoral shear force, creating stress on the cranial cruciate ligament (CrCL), which is prone to injury in dogs (Griffons, 2010; Jerram and Walker, 2003; Liu and Maitland, 2003; Slocum and Devine, 1983; Warzee et al., 2001). The execution of tibial plateau leveling osteotomy (TPLO) to create a postoperative TPA of 5–6.5° is recommended to eliminate this source of stress on the CrCL (Shahar and Milgram, 2006; Warzee et al., 2001). Previous studies have focused on TPA measurement because of the importance of its accuracy pre- and postoperatively in CrCL-deficient dogs (Caylor et al., 2001; Fettig et al., 2003; Ocal and Sabanci, 2013a; Reif et al., 2004; Ritter et al., 2007). The postoperative TPA may deviate from that planned preoperatively due to movement of the central point of the intercondylar tubercles (IC) (Duerr et al., 2008; Kowaleski and McCarthy, 2004; Kowaleski et al., 2005; Roh et al., 2020). This movement, resultant tibial axis shift (TAS), may have adverse clinical effects due to the change in location of the proximal tibial plateau segment with respect to the distal tibial and distal femoral segments (Kowaleski and McCarthy, 2004; Kowaleski et al., 2005). To avoid TAS, it is suggested that TPLO should be centered on the IC (Kowaleski and McCarthy, 2004; Kowaleski et al., 2005). However, the articular surface of the proximal tibia is moved in the sagittal plane during this procedure. The articular surface is ignored when determining the tibial axis (TA), whereas the mechanical axis of a long bone is defined as a line drawn between the centers of the proximal and distal articular surfaces (Brinker and O'Conner, 2010; Dismukes et al., 2008; Tomlinson et al., 2007). Under these circumstances, the question of whether the position of the articular surface changes in the absence of a change in the IC position during TPLO arises. In the current study, two-dimensional computer-generated models of TPLO were used to test the hypotheses that (i) TAS would be related to tibial plateau rotation, despite the centering of the osteotomy on the IC; and (ii) the postoperative TPA and the position of tibial joint surface would change with respect to TAS.

Materials and Methods

Bones of 98 dogs of 23 different breeds were obtained from the Veterinary Anatomy Department collections of Aydın Adnan Menderes and Istanbul Universities in Turkey. Proximal tibial physis fusion and the absence of gross pathological changes were determined by visual inspection; any bone not meeting these criteria was excluded from the study.

The tibiae were photographed from the medial aspect with a ruler for scale to enable measurement. Special attention was given to the standardisation of bone position, particularly superimposition of the cranial ends of the medial and lateral tibial condyles. The camera was placed at the level of the proximal aspect of the tibia, at a fixed distance from the bone, and focused on the central point of the intercondylar tubercles (Figure 1). The images were transferred to a computer in JPEG format and calibrated.

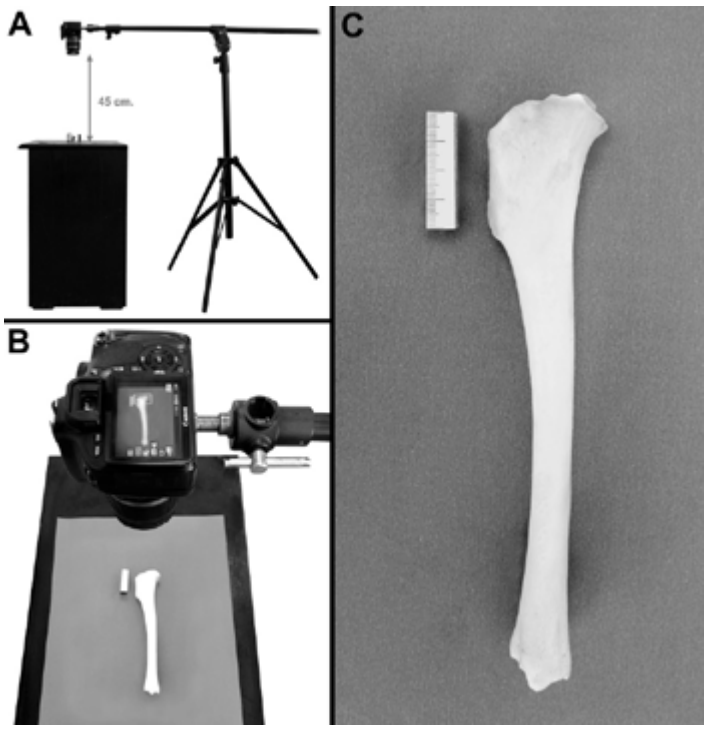

Figure 1. Showing the photographic setting system from side (A) and above (B), and the appearance of the tibia (C).

Measurements were taken according to the following procedure. First, the following reference points were marked on the calibrated photographs: the most cranial (Cr) and most caudal (Ca) points of the medial tibial condyle, to define the joint orientation line; and the IC and center of the circle that best fit the distal tibial extremity, to define the TA. The TPA was then measured in the standard manner using the TA and joint orientation line (Brinker and O'Conner, 2010; Dismukes et al., 2008). The tibial length and midshaft width were measured on each tibia. The medial condyle length was measured from the Cr to Ca points of the medial tibial condyle. The distance between the point of the TA on the medial condyle and the Cr was measured as the (cranial length of medial condyle) MCLcr (Figure 2A). Two-dimensional graphic representations of these points and axes in the sagittal plane were created using software (SolidWorks 2010, Massachusetts, USA).

TPLO was simulated for all specimens, on the 2D graphical representation. A circle with a 24-mm radius was placed on the TA at the IC, and the tibial plateau was rotated to achieve a TPA of 5° (Kowaleski and McCarthy, 2004). All measurements were retaken on these simulations, and vertical and horizontal movements of the Cr and Ca were measured using the same software. The percentages of MCLcr were calculated as a formula; MCLcr / Medial Condyle Length. The postoperative TPA was measured using the joint orientation line and the TA that the proximal point of TA defined according to the preoperative MCLcr ratio (Figure 2B). The tibial plateau rotation in mm was also calculated as; (Preoperative TPA – 5°) x 2πr / 360° (Reif et al., 2002).

Statistical analyses were performed using software (SPSS, version 20.0 for Windows; California, USA). Data are reported as means, standard deviations, ranges, and 95% confidence intervals (CIs). Paired t-tests were used to compare pre- and postoperative TPAs, MCLcrs, and percentages of MCLcr. Pearson correlation analysis was conducted to assess whether the difference between pre- and postoperative MCLcrs was related to tibial length, tibial width, medial condyle length, and/or the degree of TPA rotation. The level of significance was set to P<0.05 for all analyses.

Results

Fourteen tibiae from 7 dogs were excluded from the study because of osteophyte formation (5 dogs) and incomplete fusion of the proximal tibial physis (2 dogs). Thus, a total of 182 tibiae from 91 adult dogs were used. These specimens were from 23 breeds: German Shepherd (n=19), Anatolian Shepherd (n=13), mixed breed (n=13), Rottweiler (n=7), Boxer (n=6), Doberman Pinscher (n=5), Pointer (n=4), Cocker Spaniel (n=3), Siberian Husky (n=3), collie (n=2), Slovensky Kopov (n=2), Setter (n=2), St. Bernard (n=2); and 1 representative each of Akbash, Bulldog, Canaan, Chow Chow, Clumber Spaniel, Great Dane, Golden retriever, Malamute, Mastiff, and Shar-Pei. Forty-six (51%) dogs were female and 45 (49%) were male.

The mean tibial length was 197 ± 30.2 mm (range, 107–276 mm; 95% CI, 193–202 mm) and mean midshaft width was 16.5 ± 2.75 mm (range, 9.23–22.7 mm; 95% CI, 16.1–16.9 mm). The mean medial condyle length was 23.9 ± 3.70 mm (range, 15.1–33.9 mm; 95% CI, 23.4–24.5 mm). The mean preoperative TPA, MCLcr and percentage of MCLcr were shown in Table 1.

To obtain postoperative TPAs of 5°, tibial plateaus were rotated a mean of 18.9±3·19° (range, 11.3–26.2°; 95% CI, 18.4–19.3°). The mean rotation of tibial plateau was 7.89±1·33 mm (range, 4·72-11·0 mm; 95% CI, 7·70-8·09 mm). Cr was moved an average of 3.69±0·89 mm (range, 1.67–7.59 mm; 95% CI, 3.56–3.82 mm) distally and 0.56±0·31 mm (range, -0·22–1·57 mm; 95% CI, 0·51–0·60 mm) caudally, and Ca point was moved an average of 3.78±0·92 mm (range, 1.94–7.07 mm; 95% CI, 3.65–3.92 mm) proximally and 2.77±0·77 mm (range, 1·03–5·50 mm; 95% CI, 2·66–2·87 mm) caudally (Figure 2B). The mean postoperative TPA, MCLcr and percentage of MCLcr were shown in Table 1.

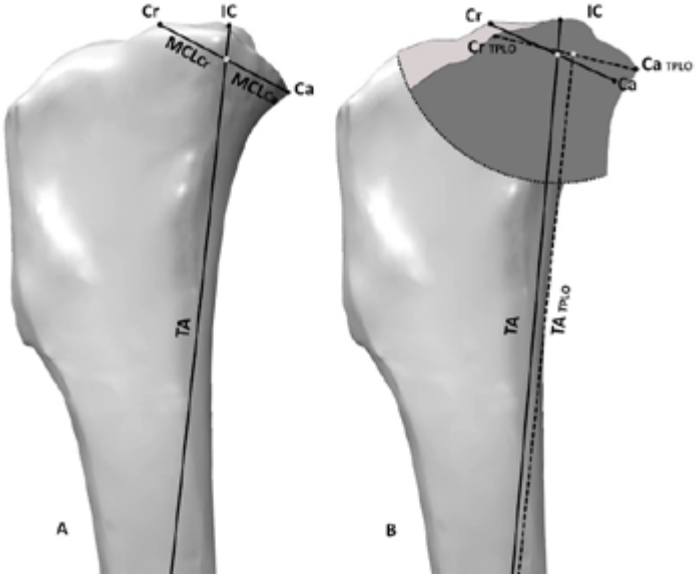

Figure 2. Depiction of tibial measurement landmarks in the sagittal plane before TPLO (A), rotation of the tibial plateau (light gray area) after TPLO (B). The center of the intercondylar tubercles (IC), cranial (Cr) and caudal (Ca) points of the medial condyle, and distances between TA and Cr (MCLcr), and TA and Ca (MCLca) are depicted, as are TPLO-induced shifts in TA (TA_{TPLO}), Cr (Cr_{TPLO}), and Ca (Ca_{TPLO}).

The mean difference between pre- and postoperative MCLcrs was 1.72±0.51 mm (range, 0.60–3.28 mm; 95% CI, 1.64–1.79 mm). Pre- and postoperative MCLcrs and percentages of MCLcr differed significantly (both P < 0.001), with mean reductions indicating a caudal shift of the TA (Figure 2B) and reduced TPA (mean, 0.52±0·15°; range, 0.23–1.05°; 95% CI, 0.50–0.54°; P < 0.001). The change in MCLcr showed a significant but fair degree of correlation with the degree of tibial plateau rotation (P<0.001, r=0.547), medial condylar length (P<0.001, r=0.495), tibial length (P<0.001, r=0.427) and midshaft width (P<0.001, r=0.402) positively.

Table 1. Comparison between the pre- and postoperative values.

Parameters	Preoperative			Postoperative			P
	Mean±SD	Range	95% CI	Mean±SD	Range	95% CI	
TPA (°)	23.9±3.19	16.3-31.2	23.4-24.3	23.3±3.09	15.9-30.7	22.9-23.8	0.001
MCLcr (mm)	12.6±2.35	6.29–18.2	12.2–12.9	10.9±2.12	5.60–16.4	10.5–11.2	0.001
Percentage of MCLcr (%)	52.5±4.71	36.6–62.0	51.8– 53.2	45.3±4.64	31.0–55.5	44.6–46.0	0.001

TPA, tibial plateau angle; **MCLcr**, distances between the tibial axis and the most cranial point of medial condyle; **MCLcr** %, its percentage.

Discussion

In TPLO, deviation from the planned TPA is likely to originate from TAS (Duerr et al., 2008; Kowaleski and McCarthy, 2004; Kowaleski et al., 2005). We observed that TAS significantly reduced the TPA, but the greatest difference between pre- and postoperative values (1.05°) was not clinically significant, as differences in TPA ≤ 2° may be ignored in dogs (Ritter et al., 2007). Among potential reasons for postoperative TPA deviation (Baroni et al., 2003; Caylor et al., 2001; Fettig et al., 2003; Ocal and Sabanci, 2013a; Reif et al., 2004), limb positioning appears to be important, as the preoperative position may be difficult to recreate accurately after an invasive operation, such as TPLO.

A mechanical axis of a long bone is defined to be drawn between the centers of the proximal and the distal joints (Allen and Mann, 2013; Brinker and O'Conner, 2010; Dismukes et al., 2007; Paley, 2002; Tomlinson et al., 2007; Wood et al., 2014). In human, the center of the knee joint is demonstrated approximately the same using a point at the center of the tibial spines or the midpoint of the tibial plateaus (Moreland et al., 1987). The former approach was adopted in dogs and the IC has been used as the proximal point of the TA in TPA measurements (Dismukes et al., 2008; Fettig et al., 2003; Headrick et al., 2007; Lister et al., 2008; Morris and Lipowitz, 2001; Sathya et al., 2014). However, there has not been any study whether the IC is corresponded to the central point of the articular surface of the medial tibial plateau or not in dogs. The pre and postoperative percentages of MCLcr were 52.5%±4.71 and 45.3%±4.64, respectively. This decrease indicated the change for the intersection of the TA and the medial tibial plateau after TPLO. The proximal point of TA should be at the central point of the medial condyle for eliminating both TAS and difference in TPA measurement postoperatively in TPLO. For similar reasons, the studies have been continued to determine the ideal osteotomy center and its applicability in TPLO; the medial collateral ligament (Ley et al., 2014) or the intersection of the tibial plateau and the TA (Kowaleski et al., 2012).

Most studies have shown that TPLO improves limb function in dogs with CrCL insufficiency (Christopher et al., 2013; Duerr et al., 2008; Nelson et al., 2013; Oxley et al., 2013; Thieman et al., 2006). However, osteoarthritis progression has been observed in stifle joints treated with TPLO (Hurley et al., 2007; Lazar et al., 2005; Shimada et al., 2020). The present study found that this procedure caused a caudal shift of the articular surface of the medial condyle, with the displacement of Cr distally and Ca proximally. Such movement of the tibial articular surface creates greater joint flexion (Boudrieau, 2009) and appears to alter the relationship between the femoral and tibial articular surfaces. An in vitro biomechanical study confirmed that the caudal shift of the articular surface of the medial tibial condyle resulted in contact with a more caudal part of the medial femoral condyle (Kim et al., 2009). This positional change after TPLO may partially explain the progression of osteoarthritis in the stifle joint, which results from a change in the load-bearing area of the articular cartilage (Andriacchi et al., 2004).

We found that TAS was correlated significantly with the degree of tibial plateau rotation, medial condyle length, tibial length, and midshaft width; all these variables except the rotation angle are unchangeable characteristics, and TPLO should avoid alteration of the tibiofemoral contact point. To prevent from TAS, the applying reduced rotation angle also contradicts the aim of TPLO, because a postoperative TPA of 5 – 6.5° is recommended to eliminate the stress on the CrCL (Nanda and Hans, 2019; Shahar and Milgram, 2006; Warze et al., 2001). This study has several limitations. TPLO was simulated, which naturally differed from the actual procedure. For example, the saw kerfs may have contributed to the observed differences in values. A circle with a 24-mm radius was applied to all tibiae, with no consideration of differences in bone size (due mostly to differences in tibial length and midshaft width) among specimens. Such standardization of the osteotomy simulation was not expected to cause significant differences in values. Furthermore, the complex structure of the tibial plateau and variations therein may be considered limiting factors for accurate tibia positioning (Ocal et al., 2013b). We tried to minimize this limitation by paying special attention to the standardization of bone position and obtaining pre- and postoperative measurements on the same images. No record of the dogs' age or soft tissue stifle joint injuries was available due to the age of the anatomical collection from which specimens were drawn. For this reason, only adult dog tibiae with no gross pathological change were included in the study.

In the sagittal plane of the tibia, the IC was located in the approximate center of the medial condyle length according to the percentage value of the MCLcr. The position of articular surface of the medial condyle was changed with respect to the IC after TPLO. The rotation of the tibial plateau during TPLO led to shift the intersection point of the TA and articular surface caudally, shortening the MCLcr by >7%. Thus, the IC would not represent to be proximal point of the TA postoperatively since the mechanical axis is a straight line connecting two joint center points.

Conclusion

The results of the present study clearly demonstrate that TPLO alters the position of the medial tibial articular surface, but not TPA clinically. Therefore, the central point of the medial condyle length is suggested to be a more suitable landmark than the IC for the proximal point of the TA in TPLO.

Acknowledgments

The authors thank to Prof. Dr. M. Kamil ÖCAL, Prof. M. Erkut KARA for all advice and support, Prof. Dr. Vedat ONAR for providing some bone materials.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Animal Health Prod and Hyg (2022) 11 (2) : 21 - 29

Animal Health, Production and Hygiene

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Research Article

The Effect of Vitamin E on Testicular Histology and Antioxidant Level in Rats Exposed to Bisphenol A

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ABSTRACT

This study, it was aimed to investigate the effect of vitamin E on testicular tissue and antioxidant levels in rats exposed to Bisphenol A (BPA). Experimental animals were divided into five equal groups. At the end of the experiment, the right testes were kept at -80 degrees to determine the antioxidant level, the left testes were fixed in Bouin's solution. In the microscopic examination, the general appearance of tubules seminiferous contours, epithelial layer, the structure of basement membrane, Sertoli cells, Leydig cells, the structure of capillaries, and tunica albuginea were examined. It was determined that the general histological appearances were similar in the control and sham groups, there was a decrease in tubule diameter in the BPA-treated group, but the seminiferous tubular epithelial layer increased. Epithelial shedding and subbasal vacuoles were detected in some tubules in the vitamin E group. Undifferentiated Embryonic Cell Transcription Factor 1 (UTF-1) positive tubule and cell counts were found to be quite high in the BPA group. The antioxidant parameter SOD value was found to be low in the BPA group and higher in the Vitamin E group. It was noted that the MDA value was higher in the BPA-applied group compared to the other groups. At the end of the study, it was determined that vitamin E applied against BPA exposure had positive effects on testicular histology and antioxidant levels in testicular tissue.

Keywords: *BPA, histology, rat, testicle, vitamin E*

Bisfenol A'ya Maruz Kalan Ratlarda Vitamin E'nin Testis Histolojisi ve Antioksidan Düzeyine Etkisi

ÖZET

Bu çalışmada Bisphenol A (BPA)'ya maruz bırakılan ratlarda Vitamin E'nin testis dokusu ve antioksidan düzeyleri üzerine etkisinin araştırılması amaçlanmıştır. Deney hayvanları beş eşit gruba ayrıldı. Deneyin sonunda sağ testisler antioksidan seviyesini belirlemek için -80 derecede tutulurken, sol testisler Bouin solüsyonunda tespit edildi. Mikroskopik incelemede tübüllerin genel görünümü, epitel tabakası, bazal membran yapısı, Sertoli hücreleri, Leydig hücreleri, kılcal damarların yapısı ve tunika albuginea incelendi. Kontrol ve sham gruplarında genel histolojik görünümlerin benzer olduğu, BPA uygulanan grupta tübül çaplarında azalma olduğu ancak seminifer tübüler epitel tabakasının arttığı belirlendi. E vitamini grubunda bazı tübüllerde epitelyal dökülme ve subbazal vakuoller tespit edildi. BPA grubunda farklılaşmamış Embriyonik Hücre Transkripsiyon Faktörü 1 (UTF-1) pozitif tübül ve hücre sayıları oldukça yüksek bulundu. Antioksidan parametre SOD değeri BPA grubunda düşük, vitamin E grubunda daha yüksek bulundu. BPA uygulanan grupta MDA değerinin diğer gruplara göre daha yüksek olduğu görüldü. Çalışma sonunda BPA maruziyetine karşı uygulanan E vitamininin testis histolojisine ve testis dokusundaki antioksidan düzeylerine olumlu etkileri olduğu belirlendi.

Anahtar kelimeler: *BPA, histoloji, rat, testis, vitamin E*

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Received Date: 03.10.2022 – Accepted Date: 03.11.2022

DOI: 10.53913/aduveterinary.1183548

Introduction

Bisphenol A(BPA), which is defined as an endocrine-disrupting chemical due to its low steroid-like properties such as polychlorobiphenyls and dioxins, was first used in pharmacy (Robins et al., 2011). Today, it has been widely used in many products such as the plastic industry, construction materials, dental fillings, and toy and beverage cans (Noda et al., 1999).

Studies have shown that BPA affects metabolism and enzyme activity in tissues, and it also disrupts the endocrine system by causing changes in hormone receptor gene activity and several receptors in target tissues (Richter et al., 2007). BPA acts in vivo by altering DNA methylation with enzyme activity and mimicking estrogen and may cause male infertility or spermatogenesis errors and metabolic disorders. Because the testicular tissue is rich in polyunsaturated fatty acids, it is easily affected by oxidative stress. Oxidative damage in the tissue and deterioration of sperm functions can cause infertility (Manfo et al., 2014).

Vitamin E is found in membrane-rich cell structures such as mitochondria and microsomes in tissues. Located between the lipid layers of the membranes, it forms the first line of defense that protects the polyunsaturated fatty acids in the structure of phospholipids from the effects of free radicals (Gupta et al., 2005). It is stated that vitamin E maintains spermatogenesis opposite oxidative stress and rise male fertilization potential (Hasanin et al., 2018). A chemical substance exposed in any period of life can have negative effects on the histological structure of the testis. This study, it was aimed to investigate the effect of vitamin E on testicular histology, spermatogonial stem cells, and antioxidant levels in testicular tissue against BPA toxicity in testicles.

Materials and Methods

Animals and experimental design

Forty adult male Wistar albino rats (240-270 g/90 days old) were used as material in the study. The rats were fed with water and fed *ad libitum* under conventional conditions in 12 hours of light/ 12 hours of darkness during the study. The material rats were divided into five groups with eight animals in each group. 1st Group (Control group): no application was made. Group 2 (Sham group): 0.5 ml corn oil was applied by gavage for three weeks. Group 3 (BPA group): BPA (Sigma-Aldrich) dissolved in 0.5 ml corn oil was applied at a dose of 10 mg/kg/day for three weeks (El-Beshbishy et al., 2012). Group 4 (Vitamin E group): Vitamin E (DL-α-tocopherol acetate, Merck) dissolved in 0.5 ml corn oil was applied at a dose of 300 mg/kg/day for three weeks (Kum et al., 2013). Group 5 (Vitamin E + BPA group): vitamin E (300 mg/kg/day) + BPA (10 mg/kg/day) dissolved in 0.5 ml corn oil was administered to the animals for three weeks. The procedure was arranged by ethical rules (Ethics Committee Approval Decision No: 64583101/2014/023).

Histological and histochemical methods

At the end of the applications, the rats were anesthetized with xylazine ketamine and killed by cervical dislocation, then their testicles were removed. While the right testes were stored at -80 °C to determine their antioxidant levels, the left testes were fixed in Bouin's solution. It is blocked in paraffin. Eight serial sections of 6 µ thickness were taken from the prepared paraffin blocks at 300 µ intervals. Crossman triple staining method and PAS (Periodic acid Schiff reagent) method was applied to determine histological, histochemical, and histometric changes on serial sections. The tubules' seminiferous contours in each section were investigated epithelial vacuolization, subbasal vacuolization, and epithelial eluded. Obtained changes were scored semi-quantitatively and subjective scoring was done according to the images. (-:None, +:Low, ++:Moderate, +++:High).

Determining histometric changes

Eight round or nearly round stages of the 7th and 8th tubules were randomly selected for each section. The diameter and height of the 7th and 8th stage tubules were measured and recorded regarding seminiferous epithelium. Interactive measurements were made with the help of the Olympus cellSens Entry image analysis program and the Olympus BX43F research microscope.

Determination of spermatogonial stem cells

The avidin-biotin staining method was used to examine spermatogonial stem cells (van Bragt et al., 2008). For this purpose, rabbit anti-UTF-1 polyclonal antibody (Rabbit anti-UTRF-1 polyclonal antibody, unconjugated, Bioss bs-12207R) was used.

Biochemical methods

The methods specified in the commercial test kit (Ref. Kot: A2300, Archem Health Ind. Co., Türkiye) were taken into account in the determination of the total protein amount and calculations. Antioxidant and oxidant parameters were determined in testicular tissue. Superoxide dismutase (SOD) analysis was applied according to Sun et al. (1988). Malondialdehyde (MDA) level in tissues was done according to the method of Yoshioka et al. (1979).

Statistical analysis

SPSS© (Statistical Package for Social Sciences) for Windows 22 (SPSS Inc., Chicago, IL, USA) package program was used for statistical analysis of the obtained data. The conformity of the data to the normal distribution was evaluated using the Shapiro-Wilk test. The difference between groups that did not show normal distribution was assessed with the Kruskal-Wallis test and the difference between groups that did show normal distribution was assessed with the one-way ANOVA (Conover, 1980).

Results

Histological findings

In the control group of rats, it was seen that the tubule's seminiferous contortus lumens were prominent, the tubules were smooth, and the spermatogenetic epithelial layer shaped a thick layer. It was observed that Sertoli cells, located between the spermatogonia, were seated on the basement membrane. Single or groups of Leydig cells located close to the blood capillaries in the intertubular space were detected. A single row of myofibroblast cells was seen around the tubules (Figure 1A-B). Capillary vessel structure was normal. Control and sham groups had similar histological appearances. In the BPA-treated group, it was noted that the diameter of the tubules decreased but the seminiferous tubular epithelial layer increased compared to the other groups. While shedding in the epithelial layer of the tubules and vacuoles located close to the basal area were observed in places (Figure 1C-D). Tubules with partially irregular contours were seen (Figure 1E). There was no difference between the groups in the basement membrane structure surrounding the tubules. It was noted that some tubules were separated from the basement membrane (Figure 1F). It was observed that germ cells were poured into the tubule lumen in any tubules (Figure 1G). Epithelial eluded and subbasal vacuoles were also observed in some tubules in the vitamin E group (Figure 1H-I). The general histological appearance was smoother in the group in which vitamin E was administered together with BPA when compared to the group with BPA. However, some tubules were found to have irregular-shaped vacuoles close to the basement membrane (Figure 1K).

Histological and histometric changes

Histological findings are given in Table 1. During the 7th and 8th stages seminiferous tubular epithelial height and seminiferous tubule diameter of the control and experimental groups are dedicated in Table 2.

When tubule diameter values were investigated, it was determined that there was a statistically important difference (P=0.023). This differences between the control group and the BPA group (P=0.012), between the sham group and the BPA group (P=0.041), between the BPA group and the vitamin E group (P=0.003), and between the vitamin E group and the BPA+vitamin E group (P=0.018) were as found to be statistically important. When evaluated in terms of epithelial height, no statisti-

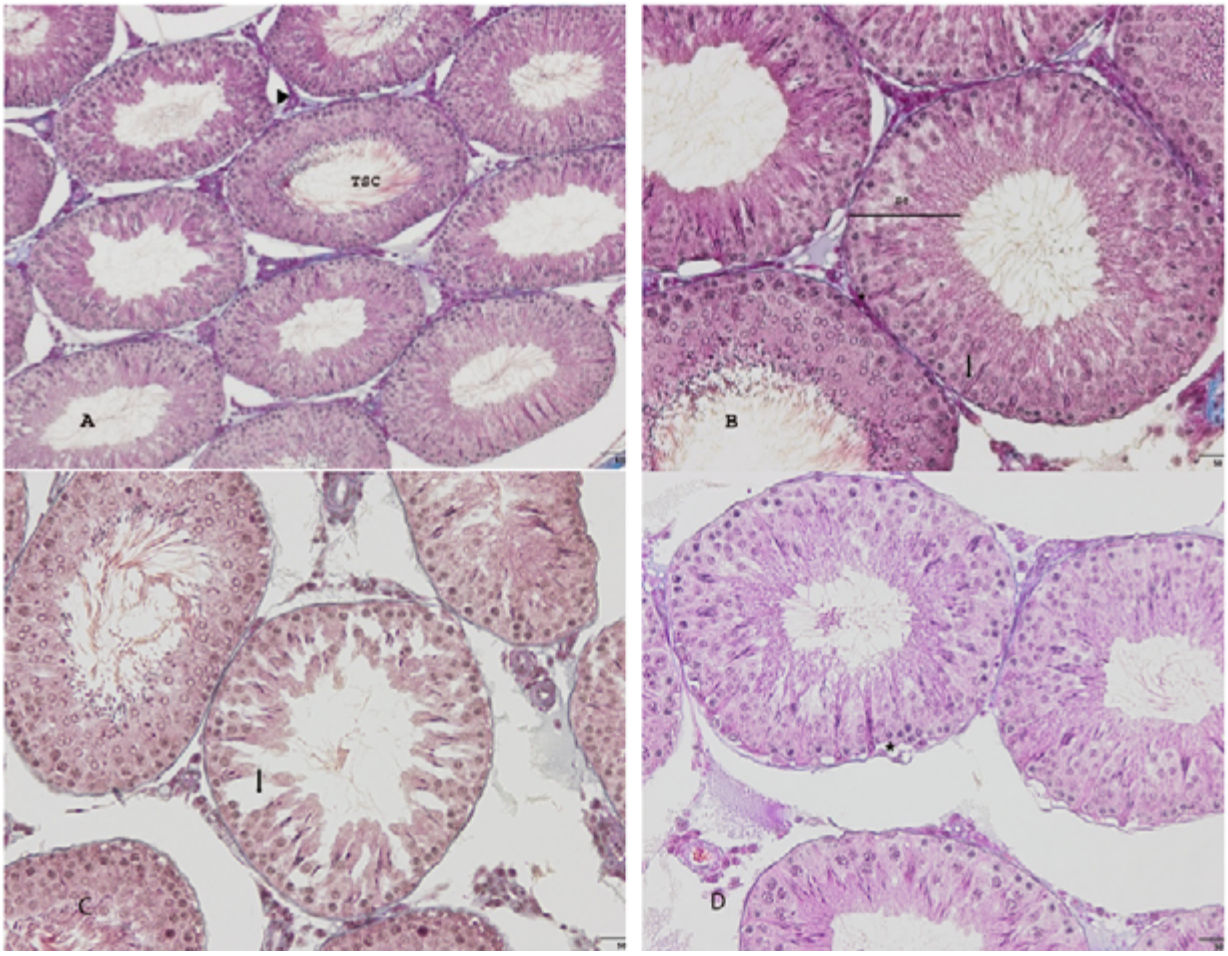

Figure1 A. General histological appearance of testicular tissue of the control group. TSC: Seminiferous tubules. Arrowhead: Leydig cells. Triple stain. B. Control group. The image of seminiferous tubules. Sc: Seminiferous epithelium. Arrow: Sertoli cell. *: Myofibroblast cells. Triple stain. C.D. The image of seminiferous tubules in the BPA group. Arrow: Epithelial shedding *: Vacuoles. Triple stain.

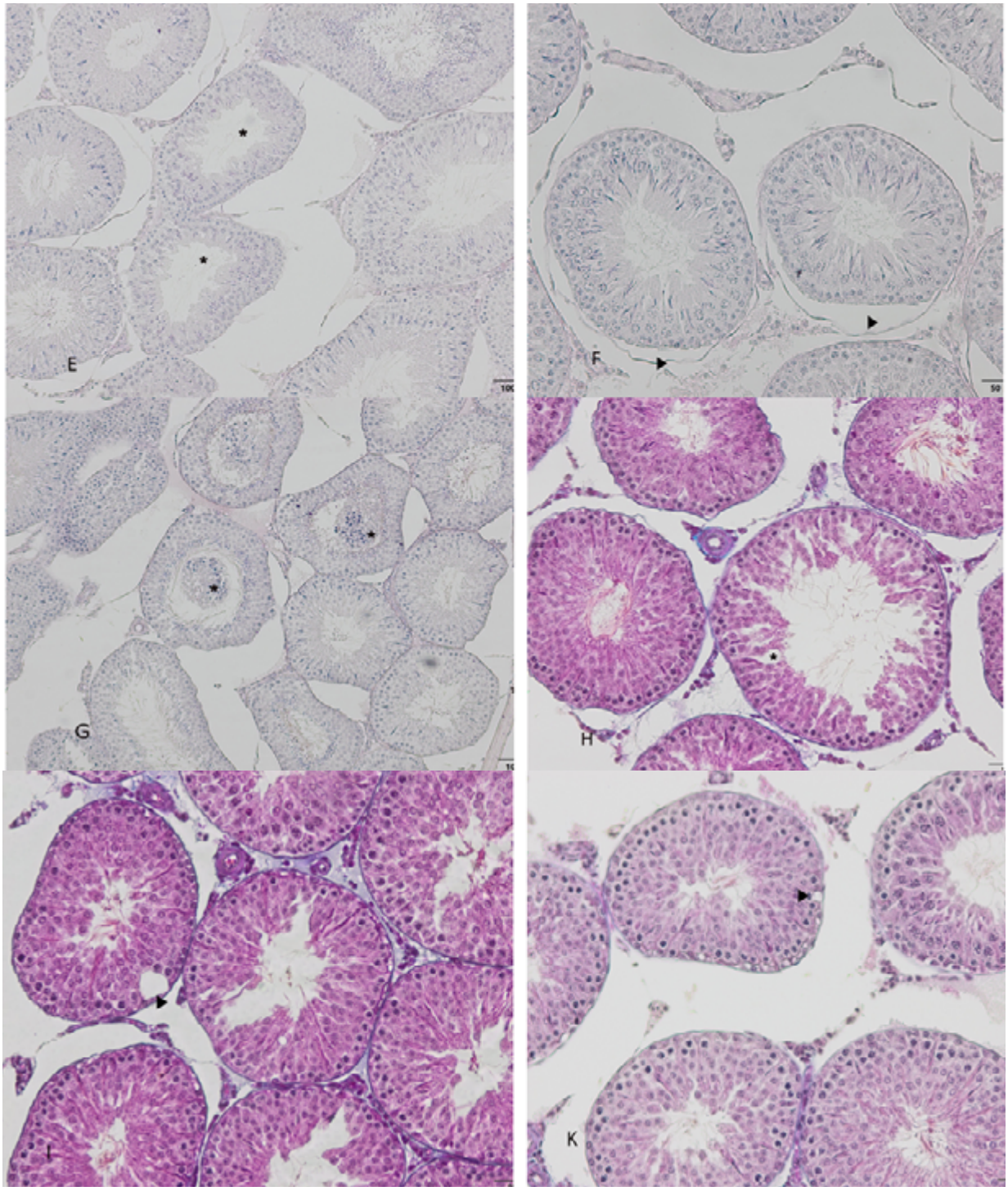

Figure1.
E.F. The image of seminiferous tubules in the BPA group. *: Tubules with irregular contours. Arrowhead: Tubules separated from the basement membrane. PAS
G. The image of seminiferous tubules in the BPA group.*: Germ cells shedding. Ta: Tunica albuginea. PAS
H.I. The image of seminiferous tubules in the vitamin E group. *: Epithelial shedding. Arrowhead: Vacuole. Triple stain.
K. The image of seminiferous tubules in the BPA group + vitamin E group. Arrowhead: Vacuole. Triple stain.

cally important difference was found between the experimental groups.

Immunohistochemical findings

The count of positive tubules and the count of positive cells were determined in three sections of each animal. The data is given in Table 3. The count of UTF-1 positive tubules and cells was found to be quite high in the group given BPA. This difference was also statistically significant. Although the positive tubule count was slightly higher in the sham group than in the control, vitamin-E, and BPA+ Vitamin-E groups, it was not statistically important. In terms of the number of positive cells, it was observed that the other groups were similar to each other except for the group with BPA (Figure 2).

Table 1. Histological changes

Groups	Subbasal Vacuolization	Epithelial Vacuolization	Epithelial Shedding	Germ cells shedding into the tubular lumen
Control	-	-	-	-
Sham	-	-	-	-
BPA	+++	++	+	+
Vit-E	+	+	-	-
BPA+Vitamin E	+	+	-	-

-:None, +:Low, ++:Moderate, +++:High

Histological changes determined as semi-quantitative were assessed with Kruskal-Wallis analysis of variance.

Table 2. Diameter of seminiferous tubules and epithelial height of seminiferous tubules.

Groups	Diameter of seminiferous tubules (µm)	Epithelial height of seminiferous tubules (µm)
Control	67.58±1.86^{ab}	276.18±11.56
Sham	67.51±1.07^{ab}	254.65±13.34
BPA	62.71±1.68^{c}	286.71±7.81
Vitamin E	69.40±1.23^{a}	282.00±10.39
BPA+Vitamin E	64.74±1.31^{bc}	260.60±10.19
P	0.023**	NS*

NS: Not significant

$^{abc:}$ Different letters in the same column indicate a statistically significant difference.

$^{*:}$ Kruskall-Wallis

$^{**:}$ANOVA

Table 3. UTF-1 positive tubule and positive cell numbers

Groups	Number of the positive tubule	Number of the positive cell
Control	7.05±1.60^{b}	6.64±1.99^{b}
Sham	9.70±2.40^{b}	8.10±2.31^{b}
BPA	15.23±0.93^{a}	21.77±4.57^{a}
Vitamin E	6.95±0.48^{b}	7.48±1.42^{b}
BPA+Vitamin E	6.66±0.48^{b}	8.29±0.87^{b}
P	0.009*	0.032*

^{ab}: Different letters in the same column indicate a statistically significant difference.

*:Kruskall-Wallis

Table 4. SOD (antioxidant) and MDA (oxidant) values in control and experimental groups.

Groups	SOD (U/mg protein)	MDA (nmol/mg protein)
Control	5.78±0.90^{b}	150.83±8.70^{bc}
Sham	4.94±0.52^{b}	172.42±9.15^{b}
BPA	1.55±0.39^{c}	206.08±4.42^{a}
Vitamin E	13.81±1.71^{a}	147.78±9.62^{c}
BPA+Vitamin E	5.25±0.97^{b}	163.63±4.61^{bc}
P	0.001*	0.001**

$^{abc:}$ Different letters in the same column indicate a statistically significant difference.

$^{*:}$ Kruskall-Wallis

$^{**:}$ANOVA

Biochemical findings

SOD and MDA values determined in testicular tissue between control and experimental groups are dedicated in Table 4.

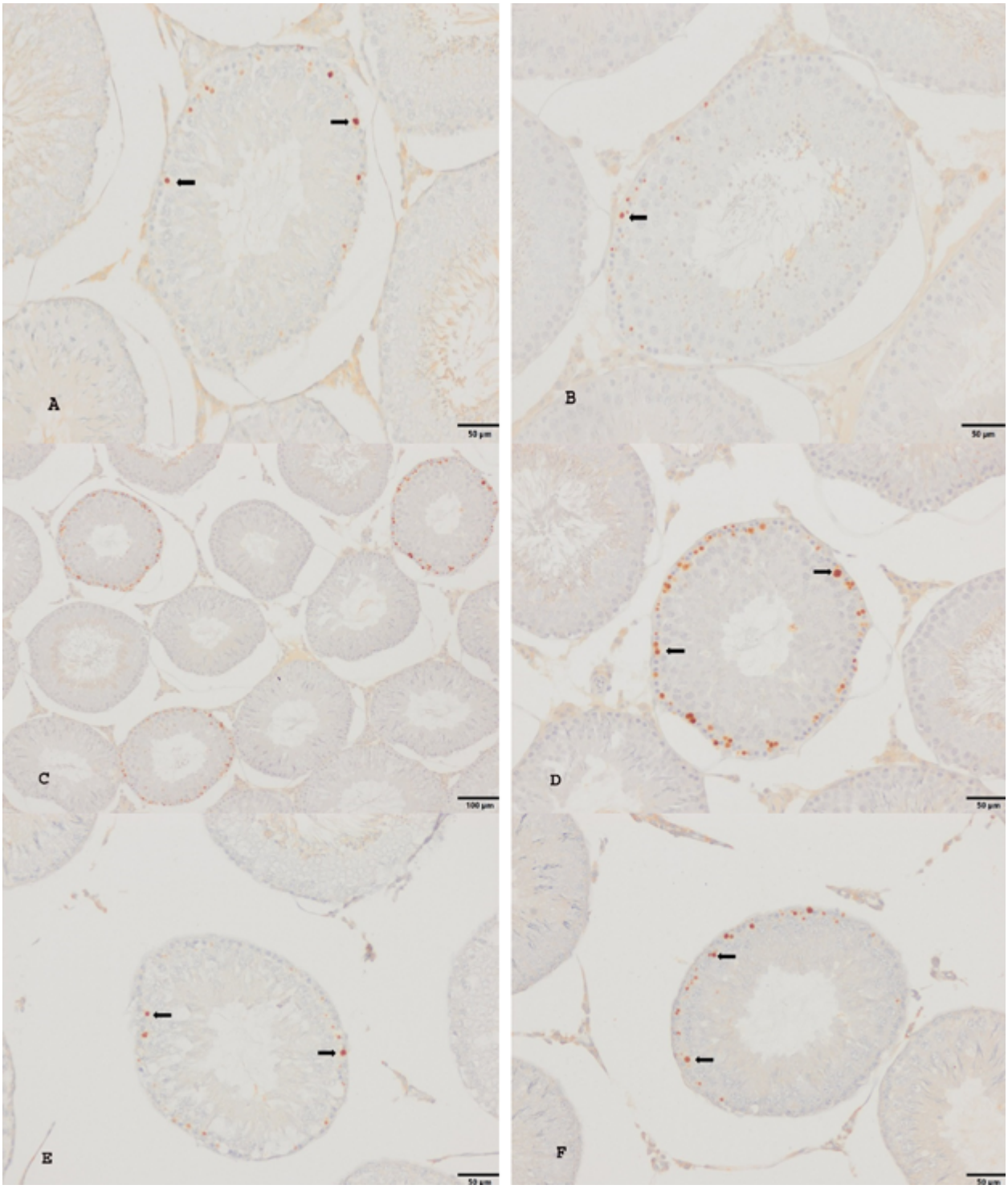

Figure 2. Spermatogonial stem cells (Arrows). A. Control group B. Sham group. C-D. BPA group. E. Vitamin E group. F. BPA+Vitamin E group. Avidin-Biotin Peroxidase.

Discussion

BPA has toxic effects on many organs such as the nervous system, brain, lung, liver, kidney, and reproductive system. In recent years, it has been stated that semen quality, decrease in sperm density, disorders in the male reproductive system, and infertility are increasing (Manfo et al., 2014). It has been reported that the seminiferous tubules lost their normal structures, the interstitium enlarged, spermatids accumulated in the tubule lumen, and degenerated Sertoli cells with pycnotic nuclei were observed in BPA-treated mice (Tolba and Mandour, 2018). It was found that BPA administered to mice impairs spermatogenesis by damaging the connection between the basal lamina of the seminiferous tubules and Sertoli cells (Tian et al., 2017). In the present study,

in the group given 10 mg/kg BPA, although severe findings were not observed, it was observed that BPA caused irregularity in some of the tubules and caused vacuoles in the epithelial layer and areas close to the basal. Epithelial detachments were also observed in some of the tubules. It can be considered that the result is related to the dose given.

The harmful effects of BPA are largely associated with its estrogenic activity (Kurosawa et al., 2002). In mammals, BPA binds and activates the two estrogen receptors ERα and ERb, although it has an affinity 10,000 times weaker than its natural ligand 17β-estradiol. It can also act with the G-protein-coupled receptor (GPR30) acting on a trans-membrane estradiol receptor (Amraoui et al., 2018). Güles et al. (2019), it was found that tubular diameter increased in BPA-treated rats checked to the control group, but this rise was not statistically important and the epithelial height in the experimental group reduced statistically important checked to the others. It has been reported that BPA given at a dose of 250 mg/kg for eight weeks reduces the height of the epithelium (Malmir et al.,v 2021). In this study, while the diameter of the seminiferous tubule decreased in the group given BPA, the height of the tubular epithelium was higher. This result may depend on the dose and time used. The Vitamin E group was similar to the control and sham groups. It has been reported that vitamin E increased the diameter of the seminiferous tubule in rats given sodium arsenide (Momeni et al., 2012). It has been shown that vitamin E increases the tubular diameter and decreased epithelial height in rats in which oxidative damage has been induced by administering para-nonylphenol (Mehranjani et al., 2009). In the presented study, it was noted that vitamin E given with BPA slightly increased the diameter of the tubule, but decreased the height of the epithelium.

Spermatogonial stem cells undergo self-renewal and differentiation to maintain the continuity of spermatogenesis throughout adulthood in men (Jung et al., 2014). UTF-1 detected in spermatogonium A during testicular development in rats can be expressed in all gonocytes in embryonic and newborn testes (van Bragt et al., 2008). It is also expressed in humans during gonadal development and in the spermatogonia of the adult testis (Kristensen et al., 2008). It has been suggested that UTF-1 is a conserved molecule of undifferentiated spermatogonia and may play a role as pluripotent transcription factor in the self-renewal of spermatogonial base cells in mammals and take part in spermatogonial regeneration (van Bragt et al., 2008; Kristensen et al., 2008). Kristensen et al. (2008) stated in their study that UTF-1 is intensely expressed in germ cell tumors, therefore germ cell tumors originate from spermatogonia. Saunders et al. (2001) reported that estrogen has a direct effect on male sex cells, while it has an inhibitory effect on Leydig and Sertoli cells, it has a stimulating effect on germ cells. UTF-1 is a protein associated with the differentiation of spermatogonial stem cells, bound to the N-terminal region of ATF-2 (Mouallif et al., 2014). UTF-1 improves transcription by acting as a co-activator to activate transcription factor-2 (ATF-2) (Fukushima et al., 1998). Vrooman et al. (2015) stated that spermatogonial stem cells were adversely affected and sperm production decreased in rats exposed to BPA. The decrease in sperm production may be due to the suppression of ATF-2 by BPA via UTF-1 (Wu and Zheng, 2013). In the present study, an intense increase in stem cells was detected in the group given BPA. This increase is thought to be due to the estrogenic effect of BPA. Increasing proof suggests that BPA may interact with testicular germ cells and reason infertility as a result of its estrogenic efficiency (Karmakar et al., 2017). Eladak et al. (2018) noticed that while BPA reduced the germ cells expressing pluripotent marker AP-2, it significantly increased the percentage of those expressing the spermatogonial marker MAGE-A4, which is highly expressed in human seminoma tumors.

In recent years, more notice has been drawn to oxidative stress, which has been identified as an important factor in male infertility and moderated by reactive oxygen species and lipid peroxidation (Agarwal et al., 2013). Studies have noticed that BPA reason oxidative stress by disrupting the balance between excessive reactive oxygen species and the antioxidant defense system in the testis (Kabuto et al., 2004). There are many protective ways and mechanisms such as SOD and GSH-Px in the cell against damage caused by free radicals (Shen et al., 2012). It is known that reactive oxygen species are produced by developing germ cells and spermatozoa during spermatogenesis. These generated radicals are cleared by a powerful antioxidant system that helps maintain the balance between the antioxidant/oxidant system. SOD is considered the first line of defense in this balance. It converts reactive oxygen molecules to H^2O^2 and O^2 using hydrogen ions. Malondialdehyde, which is widely used in the evaluation of oxidative stress, is the end product of lipid peroxidation (Bağış, 2013). A remarkable reduction in SOD levels was observed in male mice treated with BPA. Again, in the same study, it was reported that the MDA level in the testis increased (Xie and Li, 2014). It has been reported that BPA given to male rats causes a decrease in antioxidant enzymes such as GSH, GSH-Px, SOD, and catalase (El-Beshbishy et al., 2012). Amraoui et al. (2018) displayed that BPA administered remarkably increased the MDA level, while 0.5 mg/kg Selenium + 100mg/kg vitamin E administered against it decreased the MDA concentration compared to the group with BPA. In this study, the antioxidant parameter SOD value was found to be quite low in the BPA-treated experimental group, while it was found to be high in the vitamin E-administered group. There was no statistical difference between the other groups. It was observed that the MDA value was very high in the group with BPA. It is seen that the findings are compatible with the findings of other researchers.

Conclusion

In this study, it was determined that BPA had negative ef-

fects on the antioxidant levels in the testicular tissue and on the histological structure of the testis. On the other hand, it is seen that vitamin E provides an improvement in the antioxidant level and improves the histological appearance. Attention should be drawn to the use of such chemical substances against the increasing male infertility problems in our age, and new studies should be conducted to solve the problem.

Acknowledgments

This research article was summarized from the first author's master thesis. Additionally, the authors would like to acknowledge Prof. Dr. Murat BOYACİOGLU, Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Aydin Adnan Menderes University, for the evaluation of the oxidant-antioxidant parameters. The current work was financially supported by the Scientific Research Projects of Aydin Adnan Menderes University, Project No. VTF-150037.

Conflict of Interest

The authors declare that they have no conflict of interest. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Animal Health Prod and Hyg (2022) 11 (2) : 30 - 37

Animal Health, Production and Hygiene

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Research Article

Evaluation of Infrared Thermography Findings in Postoperative Follow-Up in Dogs with Pyometra

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ABSTRACT

The usability of infrared thermography (IRT) applications in the postoperative follow-up after was investigated in dogs with pyometra. The study was carried out on 14 intact female dogs undergone ovariohysterectomy, diagnosed as pyometra (Group 1, n=7) and healthy animals (Group 2, n=7). Serum C-reactive Protein (CRP) levels were measured before the operation and on the 1st, 4 th, and 7th days, postoperatively. Thermographic images from the eye and incision line were recorded, and the results were analyzed statistically. There were no intraoperative and postoperative complications in both study groups, and all dogs in Group 1 recovered uneventfully. Serum CRP levels were higher on the 0th, 4th, and 7th days in Group 1 (P<0.05), while they were at a peak level on the 1st day (P>0.05) in both groups, a decrease was observed on the 4th and 7th days (P<0.05). Postoperative CRP levels in all animals tended to decrease on the 4th and 7th days. No statistical difference between and within the eye and incision line thermographic examinations (P>0.05). While serum CRP values reached the maximum level on the 1st day after surgery in the pyometra and groups, it was seen as a milder increase due to the inflammatory reaction in the pyometra group. Due to the uncomplicated completion of the study, CRP values tended to decrease on the 4th day, and no significant difference was observed in the thermographic images**.** No statistical difference was observed in the temperature values taken from the eye and incision line in the pyometra group (P>0.05). Thermography is renowned as a non-invasive and practical application in clinical approaches. This technique may be included in the postoperative control systematic accompanied with CRP values in ovariohysterectomysed dogs due to pyometra or elective purpose.

***Keywords**: Dog, postoperative monitoring, pyometra, thermography*

Piyometralı Köpeklerde Postoperatif İzlemde İnfrared Termografi Bulgularının Değerlendirilmesi

ÖZET

Piyometralı köpeklerde kızılötesi termografi (IRT) uygulamalarının postoperatif izlemde kullanılabilirliği araştırıldı. Çalışma ovaryohisterektomi operasyonu geçiren piyometralı (Grup 1, n=7) ve sağlıklı köpekler (Grup 2, n=7) olmak üzere 14 dişi köpekte gerçekleştirildi. Operasyon öncesi (0. gün) ve postoperatif 1, 4 ve 7. günlerde serum C-reaktif protein (CRP) düzeyi ölçüldü. Göz ve ensizyon hattından alınan termografik görüntüler kaydedildi ve istatistikî yönden incelendi. Her iki çalışma grubunda da intraoperatif ve postoperatif dönemde komplikasyon gelişmedi ve Grup 1'deki tüm köpekler sorunsuz şekilde iyileşti. Serum CRP düzeyleri Grup 1'de 0, 4 ve 7. günlerde daha yüksekti (P<0,05), her iki grupta da 1. günde pik düzeyde iken (P>0,05), 4. ve 7. günlerde azaldı (P<0,05). Tüm hayvanlarda postoperatif CRP düzeyi 4. ve 7. günlerde düşüşe geçti. Göz ve ensizyon hattından alınan termografik incelemelerde gruplar arasında fark bulunamadı (P>0,05). Pyometra ve gruplarında ameliyat sonrası 1. günde serum CRP değerleri maksimum seviyeye ulaşırken, pyometra grubunda inflamatuar reaksiyona bağlı olarak daha hafif artış görüldü. Çalışmanın komplikasyonsuz tamamlanması nedeniyle 4. günde CRP değerleri azalma eğiliminde idi ve termografik görüntülerde belirgin bir fark görülmedi. Pyometra grubunda göz ve ensizyon hattından alınan sıcaklık değerlerinde istatistiksel bir farklılık gözlenmedi (P>0,05). Termografi, klinik yaklaşımlarda non-invaziv ve pratik bir uygulama olarak bilinmektedir. Bu teknik pyometra veya elektif amaçla ovariohisterektomi uygulanan köpeklerde CRP değerleri ile birlikte postoperatif kontrol sistematiğine dahil edilebilir.

***Anahtar kelimeler:** Köpek, postoperatif izlem, piyometra, termografi*

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Received Date: 13.10.2022 - Accepted Date:7.11.2022 DOI: 10.53913/aduveterinary.1174712

Introduction

Cystic endometrial hyperplasia-pyometra complex (pyometra) is a fatal disease in adult female dogs, characterized by pathological changes in the endometrium and systemic findings due to long-term exposure to progesterone and mutual interaction with bacteria (Franson and Ragle, 2003). Although the disease can be seen at almost any age, it is more common in nulliparous and old dogs, and its incidence increases with age. Different complications that will develop after the surgical approach in the treatment of pyometra are clinically significant; mortality is seen between 5-8%. Deaths are primarily due to sepsis or bacterial peritonitis, kidney and liver failures (Franson and Ragle, 2003; Jitpean et al., 2014).

In terms of preventing complications after the surgical operation and determining the prognosis, the serum concentration of acute phase proteins changes by at least 25% with the effect of pro- or anti-inflammatory cytokines. C-reactive protein (CRP), one of the crucial acute phase proteins, is produced in the liver in response to increased concentrations of proinflammatory cytokines (Eckersall and Bell, 2010). In dogs, it is increased in systemic inflammation following surgery, trauma, various infections, or neoplasia. Moreover, it is a reliable diagnostic marker for systemic inflammation (Christensen et al., 2015; Martinez-Subiela et al., 2011; Petersen et al., 2004; Slavov et al., 2011). The measurement of CRP levels in the postoperative period in dogs effectively determined the prognosis, and routine operations were used to check the healthy status (Dabrowski et al., 2009; Nevill et al., 2010; Serin and Ulutaş, 2010). It was observed that CRP concentration increased at 6 hours following the various surgical approaches (Mathon et al., 2011) and reached its maximum level at 12-24 hours (Freeman et al., 2009; Nevill et al., 2010; Saunders et al., 2009). CRP concentrations, which increase significantly during the few days following surgery (Freeman et al., 2009; Mathon et al., 2011), begin to decline due to gradual homeostasis during average postoperative recovery (Dabrowski et al., 2007). It may take one week (Mathon et al., 2011), two weeks (Zhang et al., 2013), or longer (Dabrowski et al., 2007) to return to the preoperative level.

Infrared Thermography (IRT) is a real-time, non-invasive, side-effect-free method used as an additional diagnostic tool in veterinary medicine (Franco et al., 2019; Vollmer and Mölmann, 2017). It provides the opportunity to view the changes in the skin surface temperature without touching the tissue, using a catheter or contrast material. This method allows us to view the changes in infection (Yang et al., 2018); animal welfare assessment and feed efficiency can be monitored (Cilulko et al., 2013; Martins et al., 2013; Stewart et al., 2007). In addition, IRT has been used to evaluate tissue healing by cold versus hot marking (Howe, 2006).

In the presented study, it was aimed to investigate the usability of IRT images to be taken during the first week following the operative treatment, especially in dogs diagnosed with pyometra, in the evaluation of the patient's general condition and wound healing, and to evaluate the relationship between the postoperative thermographic results and serum CRP levels.

Materials and Methods

Fourteen female dogs, 2-12 year old, were used in the study among the owned dogs brought to Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, without age or breed discrimination. During the study, the patients' perioperative general/reproductive examinations and ovariohysterectomy operations were carried out following the regulations of Aydın Adnan Menderes University Experimental Animals Application and Research Center and in line with the relevant ethics committee decision (64583101/2019/093).

In the study, it was used 14 female dogs divided into two main groups. In the first group, there were intact female dogs with variable (sanguineous/mucopurulent/purulent) vaginal discharge, polyuria, polydipsia, vomiting, diarrhea, and one or more other symptoms such as anorexia, lethargy, and depression. Moreover, confirmation of uterine infection was performed ultrasonographically (Group 1, n=7). Second group consisted healthy female dogs undergone elective ovariohysterectomy as control (Group 1, n=7). The number of dogs in groups was assigned following power test, statistically. After the anamnesis information and general examination, serum was extracted from the venous blood samples, and hemogram values were examined. In addition, the size and contents of the uterus were evaluated by transabdominal ultrasonography (My Lab-Vet 30, Esaote®-Italy). After all these examinations, dogs with a definite diagnosis of pyometra were included in the study. For the control group (Group 2) of the study, healthy female dogs (n=7) brought to our clinic with the request of elective sterilization were used and spayed via ovariohysterectomy. In addition, the general health status of the dogs allocated for the control group was checked, it was confirmed that they did not have any genital pathology after ultrasonographic examination, and attention was paid for all dogs were in the diestrus or anestrus. Venous blood samples were taken from all dogs in the study during the preoperative period; temperature, hemogram, and serum biochemical parameters (BUN, Creatinine, ALP, GPT, GGT, Total Bilirubine, Total Protein, CRP) measurements were recorded. Food and fluid intake was stopped 12 hours before the surgery. All operations during the study were performed from the median line and following standard procedures (Howe, 2006) under inhalation anesthesia using a combination of medetomidine (Medetomidine, Domitor®, Pfizer®, Finland) – isoflurane (Isoflurane USP®, Istanbul, Türkiye). After stabilization of the patients, the operation area was shaved, disinfected with iodinated antiseptic solutions, and prepared according to the necessary asepsis-antisepsis rules. Following the incision above the linea alba, both ovaries and hor-

ns were ligated and removed in routine applications, as previously stated (Hove, 2006). After the tissues were removed, an antibiotic (Unacefin®, Istanbul, Türkiye) was applied to the intra-abdominal cavity. After controlling all ligations,the closing the abdominal wall, the skin was closed with a hidden subcutaneous suture.

The operation day was recorded as the start of the study (day 0). At the end of the operation, a single dose of meloxicam (Bavet Meloxicam® Bavet, Istanbul, Türkiye) and antisedative atimepazole hydrochloride (Atipamezole, Antisedan®, Pfizer®, Finland) were administered for analgesia. Again, starting with the end of the operation, antibacterial treatment was performed with 10 mg/kg of intramuscular amoxicillin-clavulanic acid (Synulox®, Pfizer, Istanbul, Türkiye) for seven days.

Serum CRP levels were measured from venous blood samples taken on preoperative and postoperative 1st, 4th, and 7th days for patient follow-up. In addition, thermographic images were recorded by performing IRT over the eye and incision line on days 1-4 and 7. Image samples were taken from the face and operation area with an infrared Thermal camera (Flir, E6- Flir Systems®-Sweden). The changes in the temperature of the region were recorded and evaluated in terms of local and general infection. All dogs were rested for 15 minutes at room temperature in an environment without air flow, and then the thermal measurement was made at a vertical angle from an average distance of 1 meter to the operation line (Figure 1, Figure 2). After the thermographic measurement, a venous blood sample was taken, and a CRP measurement was performed with a rapid test kit (Testsealabs®, Hangzou, China).

On the relevant days, the general health status of all dogs (rectal body temperature, appetite level, pain symptoms) was recorded, and possible hyperemia, hyperthermia, and bloody-serous discharge in the wound area were investigated. In addition, the possible contribution of the thermographic findings to the postoperative patient condition and complications diagnosis were evaluated.

Statistical Package for the Social Sciences 22.0 (SPSS, IBM SPSS Statistics®, Chicago, IL, USA) package program was used for the statistical evaluation of the data obtained in this study. Distribution analysis of the data was performed using the Kolmogorov–Smirnov test. Parametric tests were applied to the parameters with a normal distribution (eye, abdomen). In this context, the Independent samples t-test was used to determine the differences between groups at each measurement time. In addition, the time-dependent variation of these parameters within the group was evaluated by repeated-measures analysis of variance, and the sampling time of the differences was evaluated with the post-hoc Bonferroni test. It was determined that serum CRP levels did not show a normal distribution despite the logarithmic transformation, and the intergroup evaluation of this parameter was evaluated with the Mann-Whitney U test. The time-dependent changes within the group were evaluated with the Friedman test. $P<0.05$ was considered significant in all analyses.

Results

During a total of 14 ovariohysterectomy operations performed during the study. Any intra/postoperative complications were not recorded as major bleeding, mesovarium, and/or uterine rupture, especially anesthesia-related complications, and related death did not occur during the monitoring period of the dogs. After one week of antibacterial and analgesic applications, the pyometra and control groups achieved the desired clinical improvement.

Postoperatively, no rectal temperature increase, opening in the incision line, seroma, or other local infection symptoms were observed in both groups. In all study material, it was observed that the surgical incision line was at a lower temperature than the surrounding tissues. However, the caudal part of the incision line was lighter in color than the cranial part. The lowest thermal temperature image was at the nipples, displayed in purple-blue. Regarding secondary infections that may occur due to suppressed immune response in the postoperative period, enlargement of various lymph nodes (mandibular, prescapular, popliteal) was not observed in our spayed

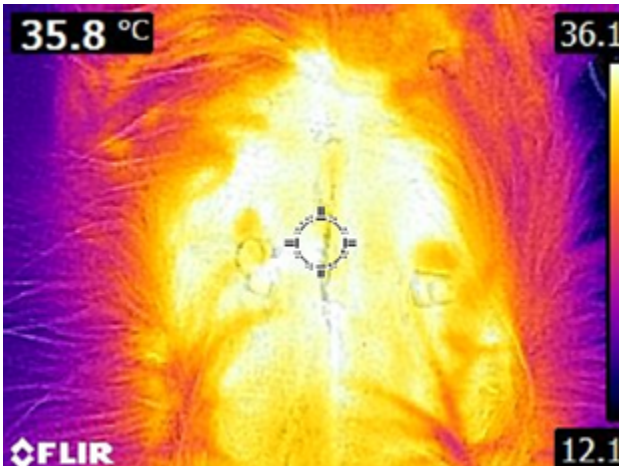

Figure 1. Incision line focused thermal image example of Group 1 (Pyometra).

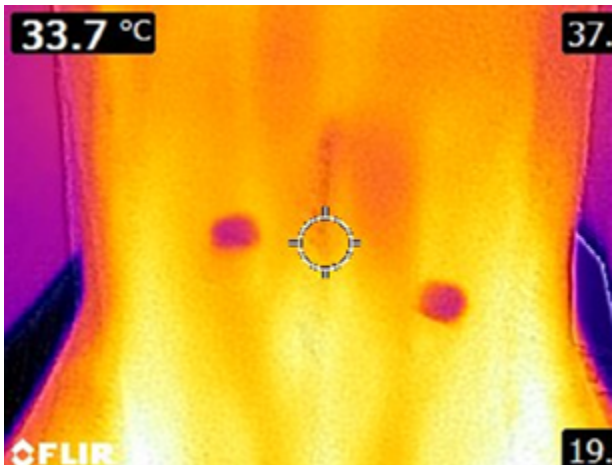

Figure 2. Incision line focused thermal image example of Group 2 (Control).

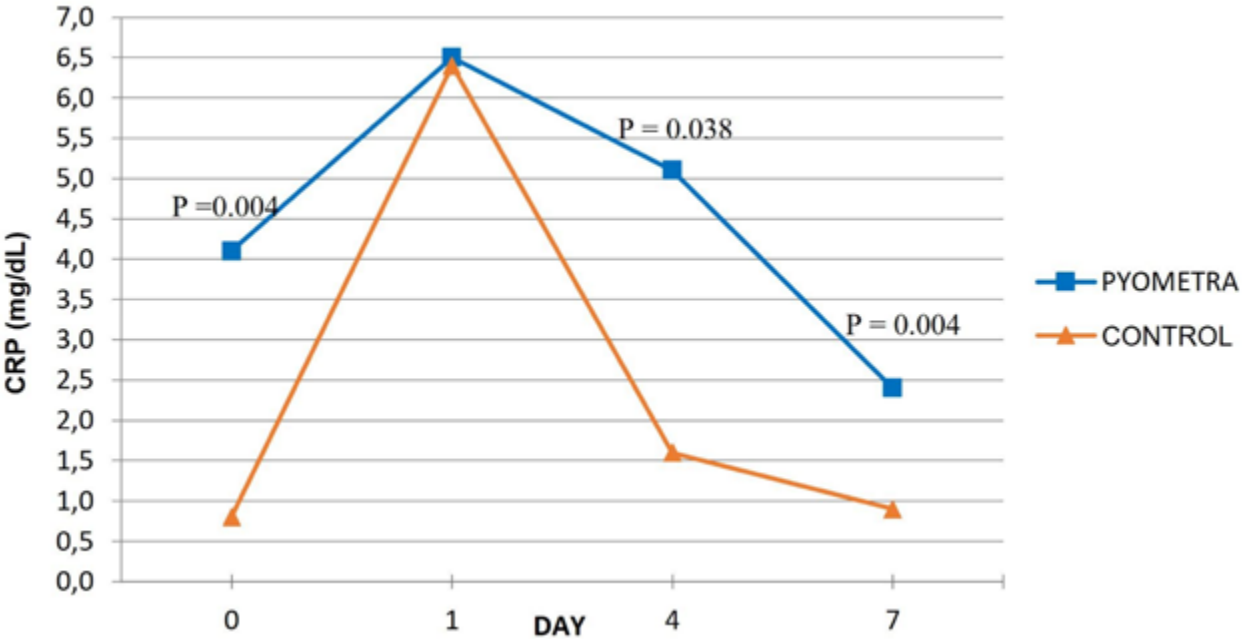

Figure 3. The mean serum CRP levels of the study groups (mg/dL).

dogs for seven days.

The mean age was 6.8 years in Group 1 and 3.7 years in Group 2, and mean rectal temperature values were measured as 39 °C in Group 1 and 38.7 °C in Group 2. No hyperthermia was observed in any animal during the postoperative 7-day period. Four patients of Group 1 had an active vaginal discharge in the preoperative period.

Accordingly, mean serum CRP values on days 0-1-4 and 7, for Group 1 were 4.1±0.95 mg/dL, 6.5±0.37 mg/dL, 5.1±0.95 mg/dL and 2.38±0.51 mg/dL; and for Group 2 were 0.84±0.25 mg/dL, 6.4±0.29 mg/dL, 1.58±±0.22 mg/dL and 0.87±0.1 mg/dL, respectively. As a result of the statistical measurements, a significant increase (P>0.05) was observed in the first 24 hours following the operation; It showed a significant decrease on the 4th day (P<0.05) (Figure 3).

CRP level of Group 1 (pyometra) was significantly higher on day 0 (P<0.01); on Day 4 (P<0.05) and Day 7 (P<0.01). However, there was no difference between the groups on the 1st day when the serum CRP levels were maximum (P>0.05). As time-dependent variation for Group 1: significant between the 1st and 7th days (P<0.01), a moderate (P<0.05) difference between the 1st and 4th days were seen. For Group 2: significant difference was found between preoperative and 1st days (P<0.05); also, between the 1st and 7th days (P<0.05). Since the preoperative CRP level of Group 2 was lower, it gave a faster and more dramatic response to the surgical intervention. Afterward, it showed a tendency to decline rapidly.

The mean values of the IRT results taken over the eye and incision line for postoperative follow-up are presented in Table 1 and Table 2. Thermal images of the eye and the incision line of both groups were recorded on days 1-4 and 7 after surgery were recorded. The values of the acquired images are shown in Figure 4 and Figure 5.

Table 1. The mean eye temperature of the study groups (°C).

Days	Group 1 (n=7) (Pyometra) X̄±S	Group 2 (n=7) (Control) X̄±S	P
1	33.72 ± 0.19	33.40 ± 0.88	0.72
4	33.88 ± 0.28	33.67 ± 0.74	0.79
7	33.21 ± 0.26	33.85 ± 0.15	0.06
P	0.21	0.77	

P<0.05: Significant difference between study groups.

Table 2. The mean incision line temperatures of study groups (°C).

Days	Group 1 (n=7) (Pyometra) $\bar{X} \pm S$	Group 2 (n=7) (Control) $\bar{X} \pm S$	P
1	35.50 ± 0.14	35.10 ± 0.15	0.21
4	35.60 ± 0.22	34.64 ± 0.57	0.14
7	35.51 ± 0.41	35.30 ± 0.21	0.65
P	0.93	0.52	

P<0.05: Significant difference between study groups.

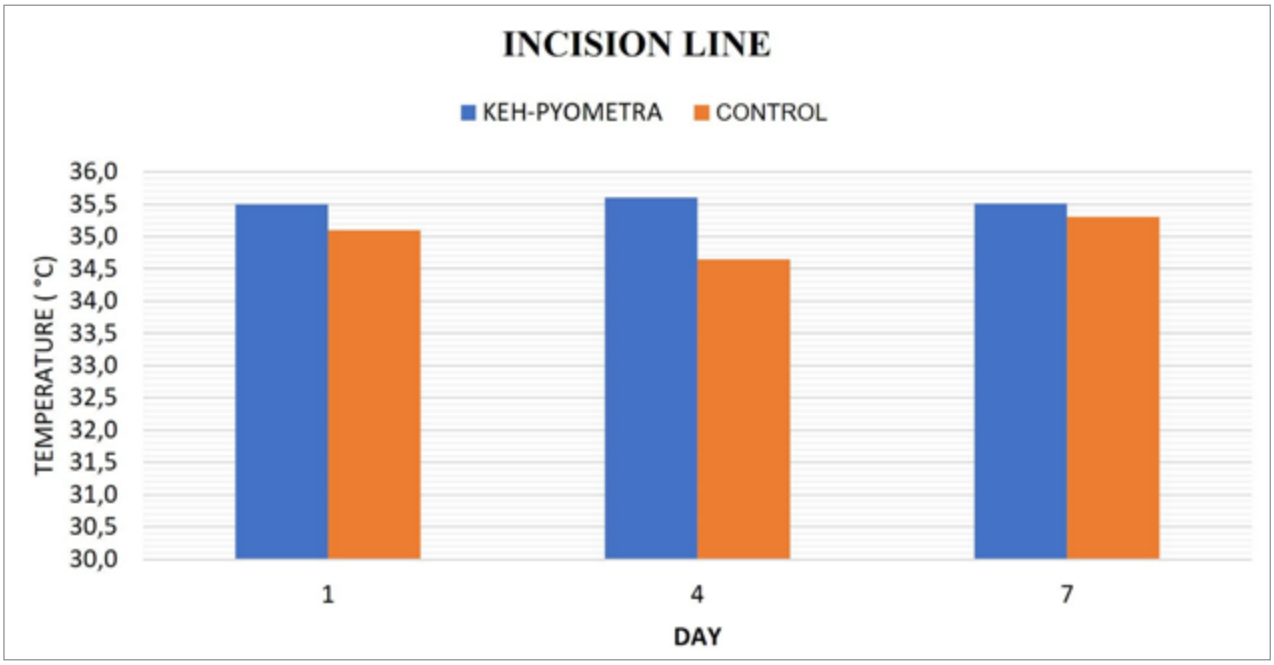

Figure 4. The mean incision line temperature of the study groups (°C).

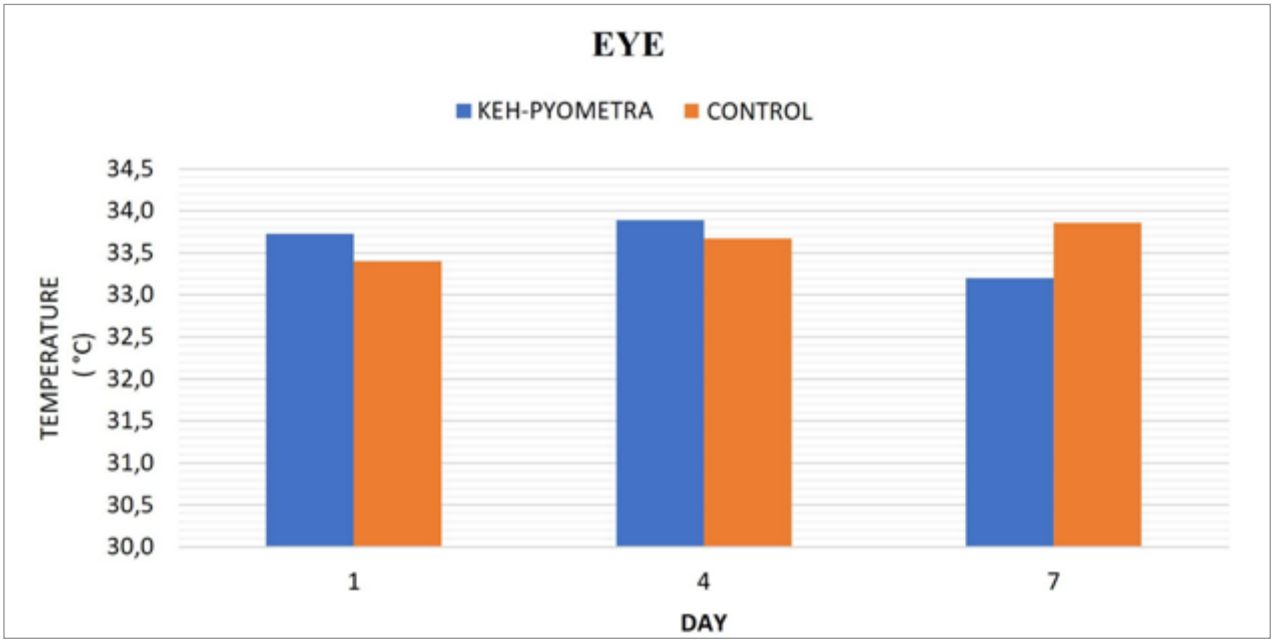

Figure 5. The mean eye temperature of the study groups (°C).

Statistically, no difference was observed in the in-group and between-group evaluations (P>0.05).

Discussion

Pyometra is a widespread phenomenon in intact adult female dogs and has an important place in the patient portfolio of small animal clinics. Medical treatment methods are tried in a minimal group, but generally, patients can be cured with radical operations (Franson and Ragle, 2003). Although ovariohysterectomy is a routine practice with well-defined borders, new applications that will determine the prognosis in the postoperative period are vital in increasing physicians' clinical success. Our literature research found no thermographic study example after pyometra operation in dogs. In this respect, it can be accepted that our study is a first in its field.

In the presented study, it was aimed to investigate the usability of IRT images to be taken during the first week following the operative treatment, especially in dogs diagnosed with pyometra, in the evaluation of the patient's general condition and wound healing and to evaluate the relationship between the postoperative thermographic results and serum CRP levels. At the end of the first 24 hours after the surgical intervention, the serum CRP level was found to be at its maximum level for both groups. Therefore, in the postoperative follow-up of spayed dogs, CRP results at about 6 ng/mL in the 1st-day controls can be a reference value for healthy groups and can be evaluated in routine applications. Serum CRP values have been evaluated as a reliable parameter for monitoring the postoperative period in dogs (Dabrowski et al., 2009; Jitpean et al., 2014). In addition, CRP is an inflammatory product marker for complications ranging from local complications to severe septic responses (Black et al., 2004; Löfqvist et al., 2018). Since it is known that using ketamine as an anesthetic causes a decrease in postoperative CRP concentration (Liao et al., 2014; Singh et al., 2020), inhalation anesthesia was preferred in our study, besides its safety in dogs with pyometra that are in older ages and have high renal dysfunction risks. In this respect, it is clear that physicians who want to follow up with CRP should be careful about the anesthetic agent they will use. Due to the CRP measurements' limited prognostic value to be made preoperatively or once afterward (Jitpean et al., 2014), periodic sampling was performed at least three times during the first week.

At the beginning of the study, the serum CRP value was higher in the pyometra group (P<0.01), and the peak value on the 1st day following the operation in both study groups is in line with the literature (Mathon et al., 2011; Nevill et al., 2010). Results in the range of 6-6.5 mg/dl on the first day were the maximum values achieved for uncomplicated cases. However, 296.6 µg/ml were seen as local complications. In that study, the maximum increase in CRP level was detected on the 4th day in the presence of redness at the wound line, hyperthermia at the incision line, and local bloody-serous discharge in the postoperative period in dogs with pyometra (Dabrowski et al., 2009). Again, the inflammatory response to the surgical intervention can be observed on the 1st and 4th postoperative days (Albert et al., 2019). In our study, the tendency to CRP levels to decrease is a typical situation in both groups in which rectal temperature and other vital values remained stable on the control days and no complications were observed. However, this decrease progressed more slowly in the pyometra group and more rapidly in the control group than in the control group. This situation is very positive in determining the prognosis, and it has been found clinically very useful to take the relevant measurements quickly, easily, and cheaply.

Postoperative wound care is one of the essential steps of clinical recovery. In animal studies, thermographic monitoring is used after burns, thrombosis formations, various malformations, oncological surgical interventions, and flap applications (Casas-Alvarado et al., 2020; Hummelink et al., 2020; Kraemer et al., 2011). Research in this area has high standardization and precision in median incision line examination results. Determining the criteria for wound control and the presence of sensitive techniques are essential in reducing complications, in this respect, quick and practical evaluations by small-sized thermal cameras are effective. In this study's results, in the abdominal images, by the literature, it was seen that the coldest points of the region are the nipples and the incision line. Again, in parallel with the same study results, the temperature increase in the caudal part of the incision line was detected in the color change in the thermal images. Gumpert Herlofson (2017) reported that the coldest points of the abdominal images were the nipples and the incision line. Meanwhile, a high temperature was detected on the caudal abdominal wall, which was thought to be associated with the increasing friction effect (Gumpert Herlofson, 2017). Since the imaging sample was evaluated from a single area along the incision line in our study, it was decided that more sensitive evaluations could be made with two samples taken from the most cranial and the most caudal point of the incision, especially in large dogs. An increase in temperature due to inflammation is expected in infective wounds, such as in diabetic foot cases (Slavov et al., 2011).

The thermal effects are seen differently. Immediately after the operation, regardless of the level of infection, decreased temperature in the region related to the accumulation of interstitial fluid rich in glucosamine, salt, and plasma proteins (Wiig and Swartz, 2012). Depending on the amount and location of fluid accumulation, the distribution of cold foci in thermal images varies. In cases without infection formation, the region's temperature rises with the formation of new capillaries and the onset of the healing process. However, in case of infection in the incision line, cold foci begin to be seen in thermography. Researchers noted that infective surgical wounds have cold areas in the first 4 days after colorectal surgery and are viewed at a lower temperature than healthy surgical wounds (Siah et al., 2019). Additionally, on the cesarean sections in obese women, temperature drops around 2

°C, which started on the second day, were interpreted as an infection (Childs et al., 2016). Thick subcutaneous adipose tissue in obese individuals but low vascularization is also effective in imaging the region as cold. In a similar study, cold areas on the 7th day after a cesarean section were accepted as a meaningful sign in terms of infection, and it was observed that a decrease of 1 °C in the temperature of the incision line increased the risk of infection three times (Childs et al., 2019). In addition, cooling is more severe in obese patients due to subcutaneous adipose tissue's high thermal insulation capacity (Zhang et al., 2013). In our study, no obese dog was in both study group, and neither skin cooling nor infection signs were observed. There was no delay in wound healing, the opening of the sutures, or open wound formation; also, postoperative vital values were within normal limits. Again, no difference was observed in response to stimuli, activity, appetite level, urination, and defecation behaviors according to follow-up anamnesis. During the establishment of the working hypothesis, the higher risk of postoperative infection in the pyometra group was expected to affect the results of the thermographic examination. Despite the lack of a statistical difference, the numerically higher temperature of the area compared to the control group can be explained by the successful use of postoperative antibiotics, where the healing process is at the desired level.

Since the study was completed without complications, there were no differences in IRT results between the pyometra and control groups. Similar conclusions have been made in previous studies, and wound thermography performed within two weeks postoperatively is not sufficient for diagnosis of infection unless significant complications occur (Gumpert Herlofson, 2017). However, it can be thought that these results may change with the development and use of more sensitive thermal cameras in the future. Although it is not statistically, higher temperature values were seen for the eye on the 1st and 4th days and the incision line on all days in the pyometra group. The eye temperature to be taken in the range of 33-34 °C at optimum room temperature will be considered normal for dogs. Due to the limited study material, possible differences might not be detected statistically. Therefore, it will be beneficial to apply a similar method to a much larger number of materials in the future. Patient follow-up via postoperative IRT seems to be a promising alternative, especially for laboratory animals and wild species having handling difficulties.

It was observed that dogs with pyometra showed significant changes in serum CRP levels, even if no complications were observed after surgical treatment. The change caused by the inflammatory reaction after surgery is more evident in elective operations. The response to surgery was relatively low in the patient group since CRP levels were higher in the preoperative period. While serum CRP decreased slower in dogs with the uneventful operation and postoperative recovery, it decreased more rapidly in healthy dogs.

Conclusion

It was possible to measure the wound area and general body temperature with IRT after abdominal operations in environments where remote monitoring is required to prevent increased stress response in the patient. IRT findings may help evaluate the patient's clinical recovery more accurately, for the level of vascularization and thus healing of surgical wounds, and for the early diagnosis of possible infections. Although IRT has not been widely used in patients' routine control procedures after sterilization operations, this technique may be included in the postoperative control systematic accompanied with CRP values in ovariohysterectomized dogs due to pyometra or elective purpose.

Acknowledgments

The authors would like to thank Aydın Adnan Menderes University Scientific Research Projects Unit for funding this research project numbered VTF-20014.

Conflict of Interest

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

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Research Article

Determination of Enterococcus Species and Antibiotic Resistance in Budgerigars

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ABSTRACT

In this study, it was aimed to determine the resistance profile by examining the prevalence and species distribution of enterococci in rectal samples of healthy budgerigars and their susceptibility to antibiotics. 100 cloacal swab samples were used in the study. Identification and antibiotic resistance of Enterococcal isolates obtained by classical methods were determined with the automated identification system. The results showed that the bacteria isolated in this study were 22 (75.86%) *E. faecalis*, one *S. uberis* (3.44%), two of each (6.90%) *E. faecium* (6.90%), *E. hirae* (6.90%), and *E. casseliflavus/gallinarum*. In general, they were suspectible to amoxicillin clavunate (96.4%), ampicillin (100%), ciproflaxacin (54.2%), levoflaxacin (60.9%), gentamicin (syn) (82.1%), streptomycin (syn) (28.6%), tigecycline (80%), vancomycin (89.3%), teicoplanin (96.4%), linezolide (96.4%), and nitrofurontion (100%), and they showed 100% resistance to cefoxitin, amikacin, gentamicin, tobramycin, clindamycin, erythromycin, trimethoprim-sulfamethoxazole (TMP-SXT), fusidic acid, and quinopuristin-dalfopuristin. The presence of Entrococcal species, which are very important in terms of zoonosis, in healthy budgerigars was revealed. In addition, the different types of antibiotic resistance found in the studies also reveal the necessity of performing antibiotic susceptibility tests in this type of infections. However, it has been demonstrated that which antibiotics will be effective in nosocomial and/or gastrointestinal infections of Enterococcal origin in budgerigars.

Keywords: *Antibiotic resistance, Enterococcus spp, budgerigar*

Muhabbet Kuşlarında Enterokok Türlerinin Dağılımı ve Antibiyotik Dirençliliklerinin Belirlenmesi

ÖZET

Çalışmada sağlıklı muhabbet kuşlarının rektal örneklerinde enterokokların yaygınlığı ve tür dağılımı ile bu türlerin antibiyotiklere duyarlılıkların incelenerek direnç profilinin belirlenmesi amaçlanmıştır. Araştırmada 100 adet kloakal svab örneği kullanılmıştır. Klasik yöntemler ile elde edilen enterokok şüpheli izolatların identifikasyonları ve antibiyotik dirençlilikleri otomatize identifikasyon sistemi yardımı ile saptandı. Çalışma sonuçlarında 22 adet (%75.86) *E. faecalis*, 2'şer adet (%6.90) *E. faecium*, (%6.90) *E. hirae*, (%6.90) ve *E. casseliflavus/gallinarum* ve 1 adet de *S. uberis* (%3.44) elde edilmiştir. İzolatlar; amoksisilin klavunat (%96.4), ampisilin (%100), siproflaksasin (%54.2), levoflaksasin (%60.9), gentamisin (syn) (%82.1), streptomisin (syn) (%28.6), tigesiklin (%80), vankomisin (%89.3), teikoplanin (%96.4), linezolide (%96.4) ve nitrofurontion'a (%100) oranlarında duyarlı, sefositin, amikasin, gentamisin, tobramisin, klindamisin, eritromisin, TMP-SXT, fusidik asid ve quinopuristin-dalfopuristine karşı ise %100 direnç göstermişlerdir. Araştırmada Zoonoz özelliği açısından oldukça önemli olan Entrokok türlerinin sağlıklı Muhabbet kuşlarındaki varlığı ortaya konmuştur. Ayrıca çalışmalarda rastlanan farklı antibiyotik dirençlilik tipleri de bu tip enfeksiyonlarda antibiyotik duyarlılık testlerinin mutlaka yapılması gerekliliğini de ortaya koymaktadır. Bununla birlikte Muhabbet kuşlarında Enterokok kökenli nozokomiyal ve/veya gastrointestinal enfeksiyonlarda hangi antibiyotiklerin kullanılmasının etkili olacağı ortaya konulmuştur.

Anahtar kelimeler: *Antibiyotik dirençlilik, Enterococcus spp, muhabbet kuşu*

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Received Date: 20.10.2022 – Accepted Date: 09.11.2022 DOI: 10.53913/aduveterinary.1192214

Introduction

Enterococci live commensally in the intestinal tract of animals and humans, soil and water (Nasiri and Hanifian, 2022). These bacteria, which are zoonotic, gain pathogenicity when the immune system of the living thing they live in is suppressed under various conditions.

These bacteria are opportunistic pathogens and part of intestinal microbiota. They may become pathogenic agents when the immune system of hosts is suppressed under various conditions (Huycke et al., 1998; Tran et al., 2022). Enterococci can survive at salt concentrations as high as 6.5%, where Gram-positive streptococci cannot grow (Gaca and Lemos, 2019). Also, they can survive high temperatures; They can survive as high as 50 °C, or even up to 30 minutes at 60 °C. They can grow optimally at 37-40 °C depending on the medium. Enterococci can also survive in environments (with some strains based on pH 10.0) that thrive best at pH 7.5, but at pHs as high as 4.8 and 9.6 (some strains based on pH 10.0) (Fisher and Phillips, 2009). They can live 40% (w/v) in bile salts that can remove other bacteria as *Streptococcus pneumoniae* (Murray et al., 2009; Teixeira et al., 2011). The extreme conditions in which enterococci can survive allow them to colonize a wide variety of sites that may be of relevance to their clinical significance (Vu and Carvalho, 2011). The ability of enterococci to withstand wide pH ranges is due to their membrane durability and impermeability to acids and alkalis, while their temperature resistance is attributed to membrane lipids and fatty acids (Fisher and Phillips, 2009). Enterococci are known to be involved in the infections of poultry, canaries and parrots (Devriese et al., 1996). It is stated that these may be infections triggered by secondary infections, and underlying viral or bacterial infections. Six species of the genus Enterococcus *(E. avium, E. cecorum, E. durans, E. faecalis, E. faecium and E. hirae)* have been associated with diseases in poultry (Christensen and Bisgaard, 2016). Of these species, *Enterococcus faecalis* is the most abundant in humans and animals, including mammals and birds. This species (*E. faecalis*) is part of the normal intestinal microflora of mammals and has likewise been reported to be the predominant intestinal microflora in poultry (Devriese et al., 2006). However, this species *(E. faecalis)* is also recognized as an opportunistic pathogen with the potential to cause clinical infections. They are also resistant to a wide variety of antibiotics and may show zoonotic properties. Today, the genus *Enterococcus* has been studied in detail from humans and animals and 38 species have been identified. Among these, the most important species as human and animal pathogens are *Enterococcus faecalis* and *Enterococcus faecium*. Researchs are also ongoing on *Enterococcus gallinarum* and *Enterococcus casseliflavus* because they are naturally resistant to vancomycin and colonize the intestinal tract (Murray et al., 2009; Wada et al., 2022). Although enterococci are low virulence bacteria, they are important agents in community-acquired and especially hospital-acquired infections. Enterococci are intrinsically resistant to many antibiotics, especially beta-lactams and aminoglycosides. They also develop resistance to some antibiotics very quickly (Franz et al., 2001). Enterococci have intrinsic resistance to many antibiotics. However, they can transfer multiple resistance to other bacteria with resistance genes in plasmids, transposons and chromosomes (Aktaş and Derbentli, 2009). Enterococci are resistant to penicillins, cephalosporins, lincosamides, trimethoprim-sulfamethoxazole (TMP-SMX) and aminoglycosides at low levels, and to polymyxins, monobactams and quinopristin/dalfopristin (Çetinkaya et al., 2000).

In Türkiye, especially the most preferred cage birds, budgerigars, the rate of being kept at home is increasing day by day. Pets are known to be able to transfer antimicrobial resistant bacteria to humans. Therefore, continuous studies are required to monitor antimicrobial resistance carried by zoonotic bacteria from the animals to human. This study was carried out to determine the resistance profile of enterococci in rectal samples of healthy budgerigars by examining the presence, prevalence and species distribution and the susceptibility of these species to antibiotics.

Materials and Methods

In our study, 100 cloacal swab samples collected from budgerigars bred or kept in cages as pet animals in Aydın province and Marmaris district of Muğla were used. Of the 100 samples collected, 50 were male and 50 were female birds. The swab samples were brought to the routine diagnosis laboratory of Aydın Adnan Menderes University Veterinary Faculty Microbiology Department under cold chain and used in the study. Since only swab samples were used in the study, an Ethics Committee Decision is not required.

Fenotypic identification

Cloacal swab samples were first subjected to the pre-enrichment procedure at 37 °C for 24 hours in Enterococcosel™ Broth. After the pre-enrichment process, a loopful of broth was taken and inoculated on 7% sheep blood agar medium and incubated at 37 °C for 24 hours. At the end of this period, gram staining was performed on the breeding colonies and catalase test was applied on those with Gram positive cocci. For this purpose, 3-5 colonies from a 24-hour pure bacterial culture grown on blood agar medium were placed on the slide with a loop. A drop of 3% H_2O_2 (hydrogen peroxide) was dropped on the slide. Strains that did not form air bubbles were considered catalase negative (Koneman et al., 1997). Bacteria with negative catalase test results were defined as *Streptococcus* spp. In order to differentiate Enterococci from these samples, they were inoculated on Enterococcocel Agar™ and incubated for 24 hours. Black colonies grown in this medium were selected and passaged on 'Tryptone soy agar' medium and pure cultures were obtained (Bilgehan, 1995). Obtained isolates were stored in Brain Heart Infusion Broth with 20% glycerol (Merck 4094) until and after the study. In order to identify the

isolated bacteria in the automated identification system, they were cultivated on Tryptone soy agar and they were grown purely. Purely obtained Enterococci suspected colonies were identified using the BD Phoenix™ fully automated identification system, Gram positive bacteria identification and PMIC/ID-87 cartridge, in which antibiotic susceptibility was determined. For this purpose, purified 24-hour fresh cultures on Tryptone soy agar were prepared in glass tubes with ID broth and a suspension according to McFarland 0.5 colony density. On-device diagnostics were performed using the BD Phoenix™ PMIC/ID87 panel kit for each sample of Gram-positive bacterial isolates. Biochemical identification data obtained from the device were evaluated.

Antibiotic resistance studies

Antibiotic susceptibility tests were performed using the BD Phoenix™ PMIC/ID87 kit of the isolates that were bacterially identified with an automated device. Purified 24-hour fresh cultures on Tryptic soy agar were suspended in glass tubes with readily available AST Broth at a McFarland colony density of 0.5. Minimal Inhibitory Concentration values were measured. In the antibiogram sensitivity profile made with the device; penicillin (P), oxacillin (OX), tobramycin (NN), cefoxitin (FOX), ciprofloxacin (CIP), clindamycin (CC), streptomycin-synergy (STS), nitrofurantoin (FM), erythromycin (E), vancomycin (V), fosfomycin (FF), gentamicin (GM), levofloxacin (LVX), amoxicillin/clavulanate (AMC), quinupristin/dalfopristin (SYN), linezolid (LZD), rifampin (RA), teicoplanin (TEC), tetracycline (TE), daptomycin (DAP), tigecycline (TGC), ampicillin (AM), fusidic acid (FA), trimethoprim/sulfamethoxazole (SXT) antibiotics were used. These antibiotic strains are available in the packages of BD Phoenix™ kits for the diagnosis of Gram-positive (PMIC/ID87) bacteria and panels for antibiotic susceptibility. Panels containing bacterial suspensions were placed in the device and bacterial diagnosis was made as well as the detection of antibiograms and sensitivity/resistance data (MIC) were obtained via the electronic system.

Results

A total of 100 samples were taken from 100 healthy budgerigars by cloacal route. *Enterococcus* spp. was isolated in 29 (29%) of 100 samples examined of these are, two (6.90%) *E. casseliflavus/gallinarum*, two (6.90%) *E. hirae*, two (6.90%) *E. faecium*, 22 (75.86%) *E. faecalis*, and one *S. uberis* (3.44%).

In this study, the distribution of 5 different species *E. faecium*, *S. uberis*, *E. hirae*, *E. faecalis*, *E. casseliflavus/gallinarum*, by species and gender is as seen in Table 1.

E. faceium species; It shows 100% sensitivity to penicillins (amoxicillin clavunate, ampicillin), fluoroquinolones (ciproflaxacin, levofloxocin), aminoglycosides [gentamycin (syn)], and streptomycin (syn) and oxazolidines (linezolid), while from gentamycin, cefhelosporins (cephelosporins), were found to be 100% resistant to macrolides erythromycin, TMP-SXT, and fusidic acid.

E. faecalis was the most common species and constituted 75.86% of the total isolates. From penicillin group antibiotics amoxicillin clavunate 95.5%, ampicillin 100%, fluoroquinolones to ciproflaxacin 50%, levofloxacin 57.1%, gentamicin (syn) 77.3%, streptomycin (syn) 13.6%, tigecycline 80%, vancomycin 95.5%, teicoplanine 95.5%, linezolid 95.5% were found to be 100% sensitive to nitrofurantoin. These *E. faecalis* strains identified at the same time showed 100% resistance against fusidic acid, tobramycin, amikacin, cefoxitin, clindamycin, gentamicin, erythromycin, TMP-SXT, and quinopuristin-dalfopuristin.

E. hirae strains showed 100% sensitivity to amoxicillin clavunate, ampicillin, gentamicin (syn), streptomycin (syn), vancomycin, teicoplanin, and linezolid. It was found to be 100% resistant to TMP-SXT.

E. casseliflavus/gallinorum strains showed 100% sensitivity to amoxicillin clavunate, ampicillin, gentamicin (syn), teicoplanin, linezolid, and 50% sensitivity to streptomycin (syn). They showed 100% resistance against erythromycin, gentamicin, fusidic acid, vancomycin, amikacin, TMP-SXT and clindamycin. *S. uberis* showed 100% resistance to gentamicin, tobramycin, and fusidic acid.

According to the data we obtained in our study, all the isolates were sensitive to levofloxacin (60.9%), streptomycin (syn) (28.6%), ampicillin (100%), ciproflaxacin (54.2%), gentamicin (syn) (82.1%), amoxicillin clavunate

Table1. Distribution by species and gender (n=29).

Factors	Gender/ Female	Gender/ Male	Total	Total %
S. uberis	1	-	1	3.44
E. hirae	2	-	2	6.90
E. faecium	-	2	2	6.90
E.casseliflavus/ gallinarum	1	1	2	6.90
E. faecalis	7	15	22	75.86
Total	11	18	29	100

(96.4%), vancomycin (89.3%), teicoplanin (96.4%), tigecycline (80%), linezolide (96.4%), and nitrofurontion (100%). However, they showed 100% resistance against gentamicin, cefoxitin, amikacin, clindamycin, erythromycin, TMP-SXT, tobramycin, quinopuristin-dalfopuristine, and fusidic acid.

Discussion

In recent years, some studies have been carried out to determine the prevalence and antibiotic resistance of Enterococcal species. In Iran, Soodmand et al. (2018) investigated the prevalence and antibiotic susceptibility of Enterococcal species among poultry and domestic birds, they collected oral and cloacal swabs from 150 caged birds and detected the presence of Enterococci in 56 of these samples. When their rates were examined, 6 Enterococci were isolated from 48 patients and 50 (49%) Enterococci from 102 healthy birds. In this study, 29 (29%) Enterococci species were isolated from 100 healthy budgerigars. It is thought that this difference is due to the presence of commercial poultry in the sample made in Iran. While all oral swabs taken from healthy animals were negative, all Enterococci growths were from cloacal swabs in parallel with our study. They emphasized that the highest Enterococcus species obtained in their research was *Enterococcus faecalis*, similar to our study. However, they identified *E. faecium* at a rate of 6.66%, similar to our study (6.90%). When the antibiotic resistance results of their studies were examined, they found that all isolates were resistant to cephalosporins in parallel with our study. However, they found that all *E. faecalis* and *E. faecium* isolates were resistant to 5 different antibiotic agents. When their sensitivity to amoxicillin was examined, it was reported that 40% of *E. faecalis* isolates and 79% of *E. faecium* isolates were found to be susceptible. However, the sensitivity of *E. faecalis* to vancomycin was reported as 29%. 22 *E. faecalis* isolates obtained in our study were found to be sensitive to vancomycin at a rate of 95.5%.

Cabral et al. (2020) investigated the distribution of Enterococcus species, their resistance to gentamicin and vancomycin in their study on Psittacine (parrot-like, curved-billed birds) birds in Brazil. For this purpose, they took samples from 126 birds and isolated *Enterococcus* species (*E. hirae*, *E. faecium*, *E. phoeniculicola*, *E. faecalis*, *E casseliflavus* and *E. gallinarum*) at a rate of 26.9%, similar to our study (29%). It was reported that the most dominant one (41.7%) among the isolated species was *E. faecalis*, similar to our study. They found high-level Gentamsin resistance similar to our study in all *E. faecalis* strains they obtained in their study. Vancomycin susceptibility was reported in two isolates (94.6%), similar to our study (95.8%). Ben Yahia et al. (2018) provide information on the possible roles of Enterococcus species found in wild birds in the spread of VanA/VanB resistance genes in their study in Tunisia. As a result of their research, the most common species in wild birds was *E. faecalis* (67.1%) as in budgerigars (75.86% *E. faecalis*). This was followed by *E. faecium* 24% and *E. casseliflavus* 8.9%. At least one (68%) of the strains of Enterococci obtained were reported to have developed resistance to the antibiotics tested. It was observed that all species obtained in their research were sensitive to ampicillin, linezolid and rifampicin in parallel with our study. The highest resistance level was found to be tetracycline (46.8%) and erythromycin (34.2%) similarly. These were followed by resistance to chloromphenicol (8.8%), gentamicin and streptomycin (2.5-3.8%), ciproflaxacin, trimethoprim sulfamethoxazole and kanamycin (12.7-21%).

Freitas et al. (2018), in a study examining the fecal microbiota of 88 Amazona aestiva parrots found in zoos in Brazil and their antibiotic resistance, *Enterococcus hermanniensis* (0.9%), *Enterococcus gallinarum* (1.7%), *Enterococcus casseliflavus*. (4.8%), *Enterococcus faecalis* (17.3%) and *Enterococcus hirae* (75.3%) species were obtained. All strains obtained were sensitive to linezoid and teicoplanin, similar to our study. However, susceptibility rates to the other 16 tested antimicrobials ranged from 0.4% to 69.3%.

In the study of Akgül et al. (2016) in chickens and seagulls, in chickens; 57.3% *E. faecium*, 4.7% *E. casseliflavus / gallinarum*, 4.1% *E. hirae*, 2.6% *E. durans*, 21.3% *E. fecalis*, in seagulls, 17.6% *E. faecium,* 8.4% *E. hirae*, 5.9% *E. casseliflavus/gallinarum*, 1.7% *E. raffinosus,* 0.8% *E. durans* and 65.5% *E. fecalis* were identified. In the study, it was found that the highest resistance in enterococci was against cefadroxil (99.5%), cefazolin (98.4%) and kanamycin (96.3%), the rate of resistance against tetracycline (18.8%) was lower than in other countries, and streptomycin (83.3%) and gentamicin (64%) determined that the resistance rates were high.

It is reported that the probability of transmission of *E. faecium* from animals to humans is low. However, *E. faecalis* poses a greater risk due to the transfer of resistance genes to virulent enterococci. (Hammerum et al., 2010). *E. faecalis* causes urinary tract infections in humans who consume and/or work with pork or poultry meat (Abat et al., 2016; Larsen et al., 2010; Poulsen et al., 2012).

The gastrointestinal tract is known as a reservoir for the exchange of genetic material by horizontal gene transfer, and the zoonotic potential of *E. faecalis* has been reported to be associated with horizontal gene transfer of genetic material encoding virulence factors and antimicrobial resistance (Werner et al., 2013). The virulence characteristics of enterococci are very important in resistance to antibiotics. Stępień-Pyśniak et al. (2019) investigated the biofilm formation ability and virulence genes of enterococci in their study with cloacal samples taken from wild birds. In the study, they stated that the increase in the hydrophobicity of enterococci species increases the aggregation substance and the ability to form biofilms accordingly. It has been shown that the hydrophobicity of *E. faecalis* is higher than that of *E. faecium* species, resulting in increased biofilm production and increased pathogenicity. In this case, it is one of the reasons explaining the high antibiotic resistance. Enterococci are intrinsically resistant to many antibiotics,

especially beta-lactams and aminoglycosides. They also develop resistance to some antibiotics very quickly. In addition to the intrinsic resistance of bacteria to many antibiotics, the acquired resistance due to resistance genes in plasmids, transposons and chromosomes and the transfer of resistance from one bacterium to another are effective in the increase of multi-resistance to antimicrobials in enterococci (Aktaş and Derbentli, 2009). Enterococci are low-level resistant to penicillins, cephalosporins, lincosamides, trimethoprim-sulfamethoxazole (TMP-SMX) and aminoglycosides, and genetically resistant to polymyxins, monobactams, and quinopristin/dalfopristin (Çetinkaya et al., 2000).

Enterococci obtained in our study were also 100% resistant to tobramycin, cefoxitin, TMP-SXT, gentamicin, clindamycin, erythromycin, amikacin, quinopuristin-dalfopuristin and fusidic acid. In 1979, high levels of gentamicin resistance began to appear in clinical isolates. This has created difficulties in the treatment of enterococcal infections. A new form of antibiotic, vancomycin, was developed in the 1990s. However, the incidence of vancomycin-resistant Enterococci (VRE) has been increasing in recent years (Woodford et al., 1998). VRE is responsible for the deaths of approximately 25,000 people each year in the United States. VRE is reported as the second most common cause of nosocomial infections (McKinnell et al., 2012). However, the prevalence of VRE in South America and Turkey is still relatively low. (Çetinkaya et al., 2000; Panesso et al., 2010). In addition to the high gentamicin resistance obtained in our study, gentamicin synergetic sensitivity is also very important. Enterococci are inherently resistant to the inhibitory and bactericidal activities of the most commonly used agents. Therefore, the recommended treatment for serious infections (ie, endocarditis, meningitis, or other possible serious infections in immunocompromised patients) includes a cell wall active substance such as penicillin or vancomycin in combination with an aminoglycoside (usually gentamicin) or sometimes streptomycin. These combinations overcome the intrinsic resistance exhibited by enterococci and achieve synergistic elimination. Therefore, while gentamicin and streptomycin are 100% resistant in many isolates, Synergetic gentamicin and streptomycin (gentamicin (Syn) and streptomycin (Syn)) give high susceptibility results.

Conclusion

The presence of Entrococcal species, which are very important in terms of zoonotic feature, in healthy budgerigars fed as pets in our homes has been revealed. Although they are harmless under normal conditions, it should be kept in mind that these bacteria can cause serious infections such as endocarditis, septicemia, urinary system infections in humans. In addition, the different types of antibiotic resistance found in the studies also reveal the necessity of performing antibiotic susceptibility tests in this type of infections. However, the data obtained in the research is also important in terms of the necessity of choosing which antibiotics in the treatment of nosocomial and/or gastrointestinal infections in budgerigars.

Acknowledgement

The study was supported by Aydın Adnan Menderes University Scientific Research Projects Unit with project number VTF-19023 and was produced from Saniye DOLHAN's Master of sciences thesis.

Conflict of Interest

The authors declare that there is no conflict of interest.

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Animal Health Prod and Hyg (2022) 11 (2) : 44 - 49

Animal Health, Production and Hygiene

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Research Article

Effects of Mucoadhesive Gel Containing Propolis on Some Biochemical and Hematologic Parameters in Rats With Experimental Periodontitis

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ABSTRACT

This study aimed to investigate the effects of propolis-containing mucoadhesive gel in experimentally induced periodontitis in rats. Propolis-containing mucoadhesive gel was prepared by using chitosan with a modified mechanical method. Thirty-five *Sprague Dawley* rats were used in the study. Rats were divided into five groups as the negative control, periodontitis + 50 mg/dL propolis, periodontitis + 100 mg/dL propolis, periodontitis + chitosan and healthy control. Experimental periodontitis was induced by placing ligatures on the inferior frontal teeth. After 11 days, the ligatures were removed, and gel applications were started. On the eighth day, blood samples were taken under anaesthesia. Haematological and biochemical analyses were performed from whole blood and serum samples. As a result of the statistical analysis, non-statistically significant decreases were determined in serum C-Reactive Protein (CRP), interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor (TNF-α) levels in the groups that were applied a mucoadhesive gel containing propolis. As a result, it was thought that mucoadhesive gel containing propolis might help treat periodontitis.

***Keywords**: Mucoadhesive gel, periodontitis, propolis, rat*

Deneysel Periodontitis Oluşturulan Ratlarda Propolis İçeren Mukoadesiv Jelin Bazı Biyokimyasal ve Hematolojik Parametreler Üzerine Etkileri

ÖZET

Çalışmada ratlarda deneysel oluşturulan periodontitisde propolis içeren mukoeadesiv jelin etkilerini araştırmak amaçlandı. Propolis içeren mukoadesiv jel kitosan kullanılarak modifiye mekanik bir metot ile hazırlandı. Çalışmada otuzbeş *Sprague Dawley* rat kullanıldı. Ratlar negatif kontrol, periodontitis + 50 mg/dL propolis, periodontitis + 100 mg/dL propolis, periodontitis + kitosan ve sağlıklı kontrol olmak üzere beş gruba bölündü. Deneysel periodontitis alt kesici dişe ligatür konularak oluşturuldu. Onbir gün sonra ligatürler çıkarılarak jel uygulamalarına başlandı. Sekizinci gün genel anestezi altında örnekler alındı. Tam kan ve serum örneklerinden hematolojik ve biyokimyasal analizler yapıldı. Sonuçların ististstiki analizinde propolis içeren mukoadesiv jel uygulanan gruplarda serum C-reaktif protein (CRP), interlökin-1 (IL-1), interlökin-6 (IL-6) ve tümör nekrozis faktör (TNF-α) düzeylerinde istatistiki önemde olmayan azalmalar belirlendi. Sonuç olarak propolis içeren mukoadesiv jelin periodonditis tedavisinde yararlı olabileceği düşünüldü.

***Anahtar Kelimeler:** Mukoadesiv jel, periodontitis, propolis, rat*

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Received Date: 07.10.2022 – Accepted Date: 14.11.2022

DOI: 10.53913/aduveterinary.1185773

Introduction

Propolis is a resinous natural product used for many purposes by mixing with wax after being collected from the secretions and buds of plants by honey bees (Popova et al., 2005). It has been shown in previous studies that the ethanolic extract of propolis has antiviral, antibacterial, antifungal, antiprotozoal, anti-inflammatory, anticarcinogenic, antioxidant, and local anaesthetic properties. Antibacterial activity of propolis has been demonstrated against gram-positive bacteria such as *Staphylococcus aureus (S. aureus)* (Valazquez et al., 2007) and gram-negative bacteria such as *Salmonella* (Orsi et al., 2005). It has been confirmed *in vivo* and *in vitro* that propolis inhibits the glycosyltransferase enzyme activity in *Streptococcus mutans* and *Streptococcus sobrinus* (Ikeno et al., 1991). Propolis induces the synthesis of insoluble glycans and inhibits the glycosyltransferase enzyme activity (Koru et al., 2007). When the researchers evaluated the antibacterial activity of propolis against some anaerobic oral pathogens, they reported that it was effective against *Lactobacillus acidophilus, Actinomyces naeslundii, Prevotella oralis, Prevotella melaninogenica, Porphyromonas gingivalis, Fusobacterium nucleatum* and *Veillonella parvula* due to the presence of aromatic flavonoid. Kujumgiev et al. (1999) reported the antibacterial activity of propolis against *S. aureus* and *Escherichia coli* and its antifungal activity against *Candida albicans (C. albicans)*. In addition, it has been reported that the co-administration of propolis with antibiotics increases their effectiveness 10 to 100 times and synergises with them. Propolis also has anti-inflammatory properties by inhibiting lipoxygenase enzymes and the production of prostaglandins. Its anti-inflammatory and analgesic properties are similar to aspirin, but it has fewer side effects (Poppe and Michelis, 1986). It also increases the production of interferon and antibodies.

Periodontitis is one of the comprehensive diseases in humans, so many studies have used experimental animals to understand its pathogenesis. The cause of tissue destruction in periodontitis is the immune and inflammatory response against pathogenic bacterial plaque. Interleukin 1beta (IL-1β) and tumour necrosis factor-alpha (TNF-α) stimulate the differentiation of osteoclast precursors and activate osteoclasts, inducing connective tissue destruction and bone resorption. Furthermore, IL-1β and TNF-α are well-researched markers of disease activity in the periodontium and act synergistically to cause bone resorption (Preshaw and Taylor, 2011).

A natural or synthetic polymer bonding to a biological substrate and keeping these two surfaces together for a long time by interfacial forces is defined as bioadhesion. If the physical surface is epithelial tissue or the mucus layer on the surface of a tissue, this attachment is defined as mucoadhesion. Mucoadhesion is a practical method for drug immobilisation or localisation (Boddupalli et al., 2010). Mucoadhesive drug release systems have advantages such as a long residence time of the drug in the region, localisation of the release system in a specific area, and an increase in the drug concentration gradient due to the intense contact of the drug with the mucosal surface. Chitosan is a bioadhesive polymer used in mucoadhesive formulations. Chitosan is a biologically cationic polysaccharide formed by combining a monosaccharide with a glycosidic bond. Due to its positively charged nature can bind very strongly to negatively charged materials such as cell surface and mucus. Although chitosan is used as a diluent, it is also used as a binder, lubricant or strong dispersant. The mucoadhesive properties of chitosan facilitate the local delivery of drugs and other substances in the oral cavity (Singh et al., 2011).

Therefore, this study aimed to investigate the effects of mucoadhesive gel containing ethanolic extract of propolis by experimentally constructing a periodontitis model. For this purpose, it was decided to examine the levels of proinflammatory cytokines IL-1, IL-6 and TNF and early inflammation marker CRP in rats with experimental periodontitis. It also aimed to demonstrate propolis's therapeutic efficacy in treating periodontitis, one of the gingival diseases, by observing haematological changes.

Materials and Medhods

Animals

This study was started with the approval of Adnan Menderes University Animal Experiments Local Ethics Committee dated 28/10/2020 and numbered 64583101/2020/104. *Sprague Dawley-type* male rats used in this study were obtained from Aydın Adnan Menderes University Veterinary Faculty Experimental Animal Production and Research Center. The study took 35 Sprague Dawley-type male rats with an average of 200-250 grams weight. During the experiment, the animals were made of transparent polycarbonate material of 420 x 260 x 180 mm in controlled rooms at Adnan Menderes University Veterinary Faculty Experimental Animals Unit with 40-60% humidity, optimum temperature (22°C), 12 hours of light and 12 hours of darkness. They were housed in cages made of stainless steel with cage tops. Animals were adapted to the experiment room before the study started. The water and feed were met *ad libitum*. The daily care of the rats was done from 10:00-12:00 every day.

Preparation of mucoadhesive gel formulation

A modified mechanical process was used to prepare the mucoadhesive gel containing propolis. Continuous mixing was done by adding 5% glycerol to 4% chitosan solution designed in 3% acetic acid. Propolis extract in ethanol was added to the gel at the determined doses (50mg/dL and 100mg/dL). All the formulations were stored in a screw-capped wide-mouthed beaker covered with aluminium foil in a calm and dark place (Partha et al., 2016).

Animals were divided into five groups as healthy control, negative control, chitosan, propolis 50 (50 mg/kg propolis) and propolis 100 (100 mg/kg propolis). Each group included 7 animals, and groups were kept in separate cages.10 mg/kg Xylazine (Rompun®, Bayer, Topkapı, Türkiye) and 100 mg/kg ketamine (Ketalar, Pfizer, Istanbul, Türkiye) were prepared for anaesthesia in periodontitis rat groups. 3/0 silk suture material was passed submar-

ginally to the inferior frontal teeth under anaesthesia. The knot was tied in the vestibule. On the 11th day, these ligatures were removed again under anaesthesia. The mucoadhesive gel containing 50 mg/dL and 100 mg/dL propolis was applied to the gingiva for seven days in the groups with periodontitis. The gel prepared with chitosan, used to prepare mucoadhesive gel, was applied to the rats in the chitosan group with the same method. No gel was applied to the negative control group. Seven rats were housed in different cages in the same experimental room without ligature and gel application as a healthy control group. Gel applications were made locally every day for seven days, starting when the ligature was removed. On the eighth day, intracardiac blood samples were collected under general anaesthesia.

Biochemical analysis

Serum IL-1, IL-6, TNF-α and CRP concentrations were measured using rat-specific ELISA kits (Bioassay Technology Laboratory, China) with an ELISA reader (Optic Ivymen System, Spain) according to the manufacturer's instructions.

Hematologic analysis

Haematological examinations were performed using the Abacus Junior Vet Hematology Cell Counter (Diatron MI Ltd, Hungary).

Statistic evaluation

SPSS21 (Statistical Package For Social Sciences 21SPSS INC., Chicago, IL, USA) was used to analyse the data obtained in the study. Whether the data showed normal distribution or not was evaluated with the Shapiro-Wilk test. While comparisons were made with the ANOVA test to the groups with normal distribution, the Kruskal-Wallis test was applied to the groups that did not show normal distribution. Results are shown as mean and standard deviation.

Results

When the groups were compared, no statistically significant difference in serum CRP levels could be determined. However, the CRP level of the negative control group was found to be higher than the healthy control group. Serum CRP levels were lower in rats treated with a mucoadhesive gel containing chitosan and propolis than in the negative control. Comparing the mean serum TNF-α concentrations, changes were not statistically significant. At the same time, the highest level was determined in the group with periodontitis without treatment. The level was decreased in the chitosan and 50 mg/dL propolis groups. It was lower in the 100 mg/dL propolis group than in the healthy control group. Mean serum IL-1 levels showed a slight increasing trend in all groups with no statistical significance compared to the healthy control. When the mean serum IL-6 levels were compared, no statistical difference could be found in the groups treated with healthy control negative control chitosan, 50 mg/dL and 100 mg/dL propolis.

A statistically significant difference was determined in the hemoglobin and PCT levels. In contrast, among other parameters, no statistically significant difference was observed between the healthy, negative control and chitosan and propolis-containing groups. While the hemoglobin level was higher in the negative control and chitosan gel group than in healthy rats, it was observed that it tended to decrease in the group treated with propolis-containing gels. The blood PCT level was significantly lower in rats with periodontitis that did not receive any treatment than in the healthy and treatment groups (Tables 1 and 2).

Discussion

Animal models of periodontal disease are essential in developing the scientific basis for understanding pathological processes (Graves et al., 2012). Especially rodents and rats are suitable models for experimental periodontal research (Struillou et al., 2010). The structure of the dental gingival region is similar to that observed in humans with shallow gingival sulcus and attachment epithelium to the tooth surface (Lonel et al., 2015). Some pathways in experimental periodontitis differ from human chronic periodontitis progression. Because in the model created by placing the ligature, bacterial accumulation and periodontal tissue destruction show an acute process. Despite this, it is widely used (Aral et al., 2015). Propolis antibacterial, antioxidant, antifungal, antiviral, anti-inflammatory, tissue regenerative, and wound-heal-

Table 1. Mean serum CRP, TNF-α IL-1 and IL-6 concentrations (means±standard deviation) ($\overline{X}$ ±S) in experimental periodontitis-induced and healthy control group

	Healty ($\overline{X}$ ±S)	**Nega ve Control** ($\overline{X}$ ±S)	**Chitosan** ($\overline{X}$ ±S)	**Propolis 50** ($\overline{X}$ ±S)	**Propolis 100** ($\overline{X}$ ±S)	**P**
CRP (ng/mL)	1.22±0.52	1.78±0.30	1.55±0.53	1.24±0.51	1.41±0.52	NS
TNF-α(ng/mL)	200.57±95.70	293.25±141.00	221.84±23.03	244.41±91.83	180.36±81.18	NS
IL-1 (pg/mL)	28.36±3.96	31.05±5.49	29.56±4.60	32.64±2.30	32.18±6.96	NS
IL-6 (pg/mL)	2.56±0.36	2.37±0.67	2.83±0.22	2.43±0.34	2.87±0.39	NS

NS: Non-significant

Table 2. Mean serum hematologic parameters means±standard deviation ($\bar{X}$ ±S) in experimental periodontitis-induced and healthy control group rats.

	Healty ($\bar{X}$ ±S)	Nega ve Control ($\bar{X}$ ±S)	Chitosan ($\bar{X}$ ±S)	Propolis 50 ($\bar{X}$ ±S)	Propolis 100 ($\bar{X}$ ±S)	P
WBC	9.63±3.23	11.27±3.47	8.95±3.18	9.35±1.80	9.78±2.50	NS
LYM	7.95±2.64	9.72±3.04	7.31±2.84	7.06±1.88	7.68±1.91	NS
NEU	1.34±0.66	1.37±0.55	1.33±0.50	1.92±1.57	1.63±0.87	NS
%LY	82.75±5.18	73.18±31.98	80.95±4.19	75.91±16.06	79.60±15.57	NS
%NE	13.58±3.59	12.11±2.60	15.90±5.57	20.56±16.52	16.28±5.83	NS
RBC	9.91±4.63	9.85±0.72	9.11±0.85	8.01±0.37	8.56±0.68	NS
HGB	13.56±1.39^{a}	15.25±0.48^{b}	15.85±1.00^{b}	14.30±1.21^{ab}	14.71±0.66^{ab}	*
HCT	62.17±41.17	137.16±204.76	49.72±2.89	43.83±3.72	47.33±3.52	NS
MCHC	27.13±9.66	28.53±2.40	31.83±0.72	32.68±1.98	31.14±1.70	NS
PDWc	18.08±6.03	16.20±0.55	16.20±0.67	16.20±0.50	15.90±0.69	NS
PCT	0.3±0.06^{a}	0.58±0.06^{b}	0.79±0.11^{ab}	0.76±0.13^{ab}	0.78±0.30^{ab}	*
MPW	7.04±0.16	7.30±0.49	7.05±0.64	6.98±0.43	7.07±059	NS
PDWC	33.16±0.35	32.81±2.55	33.46±2.34	32.75±1.04	32.65±1.10	NS

ab: Statistical difference between groups with different letters in the same column is significant. *: P<0.05
NS: Non-significant

ing effects allow its clinical use (Eroğlu et al., 2004). Much research has been done on propolis for the mouth, and it is widely used safely. Experiments have been done on periodontitis, gingivitis and caries in dentistry (Koo et al., 2000; Skaba et al., 2013). It has been reported that the solvent used affects the antimicrobial activity of propolis. While glycerine solutions have little inhibitory effect on bacteria, ethanol solutions create an excellent inhibitory effect against bacteria and yeasts (Castaldo and Capasso, 2002).

IL-1, IL-6 and TNF-α stimulate the differentiation of osteoclast precursors and activate osteoclasts, inducing connective tissue destruction and bone resorption (Farquharson et al., 2012; Dietrich et al., 2013). These cytokines are well-researched markers in periodontal disease activity and act synergistically to induce bone resorption (Dietrich et al., 2013; Olsen, 2015). For this reason, this study aimed to show the effect of propolis in the form of mucoadhesive gel by examining the changes in these cytokines. There was a decrease in the TNF level in the gel-applied groups, but the reductions were not statistically significant. It was observed that it was lower than the healthy control, especially in the gel group containing 100 mg of propolis.

However, IL-1 and IL-6 levels did not cause a significant change when the healthy control and experimental groups were compared. Aral et al. (2015) reported that mean plasma IL-1b levels in rats with diabetes and periodontitis increased in untreated groups and decreased in propolis treatment groups. However, the differences were not statistically significant in this study either. Nishihara et al. (2009) reported that serum TNF-α levels increased in mice with experimental diabetes and periodontitis, and then decreased on the 3rd day. Takano et al. (2010) similarly induced periodontitis in diabetic mice and reported that cytokine levels increased significantly. These differences in the studies were thought to be due to the differences in the methods of inducing periodontitis and the duration of the study.

The relationship between CRP and periodontitis has received significant attention because of the link between periodontitis and cardiovascular disease (Paraskevas et al.,2008; Bansal et al., 2014). It is common to use CRP as a marker of the relationship of periodontitis with other systemic diseases (Hajishengallis and Chavagis, 2021). The serum CRP level was higher in the negative control group, which did not receive treatment. The gel containing propolis decreased in the treatment groups, but the serum CRP levels were not statistically significant. In a meta-analysis review evaluating studies on periodontitis and CRP levels, it was reported that there is a correlation between serum CRP levels in patients with periodontitis who are not systematically healthy. It has been reported that patients with periodontitis have high CRP levels, but systemic disease causes a more severe increase in CRP. In addition, it has been reported in the literature reviews that aggressive forms of periodontitis cause a more severe increase in CRP levels. Haematological changes in

patients with periodontitis were generally recorded as high WBC, high neutrophil level, low sedimentation rate and PCV (Bothello et al., 2020). This study determined that the WBC and lymphocyte count increased in the rats with experimental periodontitis compared to the healthy control and tended to decrease in the treatment groups. Although there was no statistical difference between the changes, these results were compatible with previous studies. The decrease in PCT levels was statistically significant between this study's healthy control and untreated negative control group. The stimulus in the gingiva is characterised by leukocytes rich in inflammatory infiltrate, which can then be excreted into the systemic circulation (Ryder, 2010; Hirschfeld, 2014). Alternatively, it can stimulate the bone marrow to produce more inflammatory cells chronically through continuous local inflammation and bacterial interaction (Belkaid and Hand, 2014). In addition, periodontal bacteria can invade periodontal tissues through the ulcerated epithelium and trigger a systemic response to counter harmful effects.

Conclusion

This study showed that propolis might contribute to preventing tissue destruction and healing by reducing the systemic manifestations of inflammation caused by periodontal diseases. However, more detailed studies are needed to examine the effects of propolis applied as a mucoadhesive gel. In the new studies to be planned, different periodontitis induction methods, increasing the number of subjects and trying various dose applications will improve the data on this subject. Propolis has an important place in pharmaceuticals. Applying propolis in mucoadhesive gel form is important as a new approach. In future studies, it is predicted that propolis can be more effective in this form by examining the physicochemical properties of the gel and standardising it.

Acknowledgements

This study was summarised by the first author's Master Thesis. This study was supported by Aydın Adnan Menderes University Research Foundation (VTF-21025).

Conflict of İnterest

The authors declare that they have no conflict of interest.

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Animal Health Prod and Hyg (2022) 11 (2) : 50 - 55

Animal Health, Production and Hygiene

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Review

Intestinal Aquaporins

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ABSTRACT

Aquaporins (AQPs) are integral, hydrophobic, and transmembrane proteins that facilitate passive transport of water depending on the osmotic pressure on both sides of the cell membrane. Of these channel proteins, those that are permeable only to water are called “classical aquaporins”, those that allow the passage of small molecules such as glycerol in addition to water are called “aquaglyceroporins”, and those with different structures and cellular distributions are called “super aquaporins”. Aquaporins have a great role in the gastrointestinal tract as well as in the whole organism. Transepithelial transport of fluid in the intestine occurs spontaneously, either by paracellular or cellular routes, or both. The paracellular pathway is mediated by tight junctions in the intestinal epithelium and their passage is regulated based on the size and load of substances, while the cellular pathway is passive diffusion mediated by aquaporins and co-transporters. Among them, aquaporins are the major cellular pathway for bidirectional fluid transport in the gut. Aquaporins have important roles in the gut. Based on these roles, information, and research on whether aquaporins can be regulated by drugs and dietary supplements to increase intestinal health and improve their functions is increasing day by day. In this review, the functions of aquaporins in the intestinal tract, their situations in intestinal diseases, and the drugs and dietary supplements used for the treatment of these diseases are discussed together with current studies.

***Keywords**: Aquaporin, gut health, intestinal system*

ÖZET

Akuaporin (AQP)’ler hücre membranının her iki tarafındaki ozmotik basınca bağlı olarak suyun pasif transportunu kolaylaştıran integral, hidrofobik ve transmembran proteinlerdir. Bu kanal proteinlerinden sadece suya geçirgen olanlara “klasik akuaporin”, suya ek olarak gliserol gibi küçük moleküllerin geçişine izin verenlere “akuagliseroporin” ve yapıları ile hücresel dağılımları diğerlerinden farklı olanlara ise “süper akuaporin” denilmektedir. Akuaporinler tüm organizmada olduğu gibi gastrointestinal kanalda da büyük role sahiptir. Bağırsakta sıvının transepitel taşınması ya paraselüler veya selüler yollarla ya da her ikisi ile spontane olarak gerçekleşmektedir. Paraselüler yola intestinal epiteldeki sıkı bağlantılar aracılık eder ve maddelerin boyutları ile yükleri temel alınarak geçişleri düzenlenirken, selüler yol ise akuaporinler ve ko-transporterlerin aracılık ettiği pasif difüzyon şeklinde olmaktadır. Bunların arasında akuaporinler, bağırsakta iki yönlü sıvı taşınması için majör selüler yoldur. Akuaporinlerin bağırsakta önemli rolleri bulunmaktadır. Bu rollerine dayanarak akuaporinlerin, bağırsak sağlığını artırmak ve fonksiyonlarını geliştirmek için ilaçlar ve diyet takviyeleri tarafından düzenlenip düzenlenmeyeceklerine ilişkin bilgi ve araştırmalar her geçen gün daha fazla oranda artmaktadır. Bu derlemede akuaporinlerin intestinal kanaldaki fonksiyonları, disregülasyonları ile bu disregülasyonların sağaltımı amacıyla kullanılan ilaçlar ve diyet takviyeleri, güncel çalışmalar ile ele alınmıştır.

***Anahtar kelimeler**: Akuaporin, bağırsak sağlığı, intestinal sistem*

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Received Date: 05.09.2022 – Accepted Date: 20.10.2022

DOI: 10.53913/aduveterinary.1171043

Introduction

Aquaporins (AQPs) are integral, hydrophobic, and transmembrane proteins responsible for the passive transport of water in the direction of the osmotic pressure on both sides of the cell. The aquaporins responsible only for water transport are called "classical aquaporins". Studies have shown that aquaporins are also involved in the transport of small and uncharged molecules such as glycerol, urea and ammonia, in addition to water, in the direction of the concentration gradient. And they were called "aquaglyceroporins". There are also "super aquaporins", which are considered as a separate family due to their structural and permeability differences and very low homology with other aquaporins. Classical aquaporins include AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, and AQP8; aquaglyceroporins include AQP3, AQP7, AQP9 and AQP10, and super aquaporins include AQP11 and AQP12 (Li and Wang, 2017). Why are aquaporins essential in organisms? Because about 70% of the living organism is water. Therefore, to provide homeostasis, transport of water, solutes, ions, electrolytes, proteins, and nucleic acids between intracellular and extracellular fluids is crucial. Aquaporins play a major role in this regard (Brown, 2017). The essential function of aquaporins is to intercede the transmembrane transport of small solutes, glycerol, and water, to regulate the absorption and secretion of water in the intestine, and to maintain the balance and homeostasis of intestinal fluid movement (Fischbarg, 2010). In addition to these functions, aquaporins are also required for the proliferation and migration of immune cells. Thus, they mediate the intestinal immune response and participate in the regulation of natural host defense at the cell membrane level (Lv et al., 2022). Aquaporins have a primary role in water transport in the intestinal tract since the cell-cell connections in the intestinal mucosa are shaped by tight junctions (Laforenza, 2012). Nearly all discovered aquaporins are commonly found in the large and small intestines of mammals. Besides their normal physiological functions, aquaporins are closely related to intestinal diseases such as constipation, diarrhea, colitis, and colorectal cancer. There have been many studies targeting aquaporins for the development of drugs used in the treatment of intestinal diseases. The results show that drugs in which regulates the expression of aquaporins can improve the intestinal health of mammals (Lv et al., 2022).

The distribution of aquaporins in the gastrointestinal tract is determined by their functions. For example, AQP3 and AQP4 are basolateral water channels more expressed in secretory epithelium (e.g., stomach), while apical water channels are more expressed in absorbent epithelium (e.g., small intestine). Apical and basolateral aquaporins, which are involved in both reabsorption and secretion of water, are expressed in the colon (Laforenza, 2012). Almost all discovered aquaporins are abundant in the small and large intestinal tissues of mammals and contribute to many physiological functions depending on their localization (Lv et al., 2022).

Functions of intestinal aquaporins

The main function of aquaporins is to mediate the transmembrane transport of glycerol, water, and small solutes, and to maintain the balance of intestinal fluid movement and intestinal homeostasis (Fischbarg, 2010). In addition to these functions, aquaporins are also required for the migration and proliferation of immune cells (Lv et al., 2022).

AQP1: It has been reported to be expressed in the mucosa of the ileum and endothelial cells of submucosal capillaries (Mobasheri and Marples, 2004) and endothelial cells of porcine small intestinal villi (Jin et al., 2006). In addition, it is thought that AQP1 may also be required in the digestion process of fats due to its location in the endothelial cells of the central lymphatic channels of the small intestinal villi, where chylomicrons are produced (Zhu et al., 2017).

AQP2: It is believed to be expressed in the rat colon (Chen et al., 2016; Guttman et al., 2007).

AQP3: According to a study, the most important and common aquaporin in the rat colon is aquaporin 3 (Ikarashi et al., 2011). Various studies have shown that AQP3 is found in the colonic mucosa (Cao et al., 2014; Thiagarajah et al., 2007) and jejunal villus epithelial cells (Zhang et al., 2017). It was observed that exposure to AQP3 inhibitor ($HgCl_2$ and $CuSO_4$) for more than one hour increased the water content in the faeces up to 4 times compared to the controls and a fulminant diarrhea was formed (Zhu et al., 2017).

In the trinitrobenzene sulfonic acid (TNBS)-induced colitis model, AQP3 expression was found to be down-regulated together with AQP8, and intestinal inflammation and damage were also shaped. In rats with inflammatory bowel disease, AQP3 expression increased in the adaptation process after small bowel resection and development of intestinal functions. This finding indicates that AQP3 may have a function in the pathogenesis of inflammatory bowel disease (Zhao et al., 2014; 2016).

In a sepsis-induced mucosal injury model, AQP3 expression was downregulated as result of sepsis-induced intestinal damage and inflammation (Zhu et al., 2019). In addition, trefoil factor peptides that stimulate epithelial cell migration to limit luminal damage in acute gastrointestinal mucosal damage and inflammation and prevent further damage and inflammation in chronic conditions (Aihara et al., 2017), increase the level of AQP3 expression to achieve epithelial cell migration. It gives the cell the ability to deform, and thus, epithelial cell migration towards the damaged area is ensured (Marchbank and Playford, 2018).

Cell damage model shows that AQP3 affects the H_2O_2 permeability of the membrane, inducing the production of actin-derived lamellipodia, thus contributing to endothelial cell migration and damaged epithelium repair (Thiagarajah et al., 2017).

AQP4: It has been reported to be expressed in the mouse colon and ileum (Jiang et al., 2014; Wang et al., 2000), as well as in the basolateral membrane of ileal epithelial cells (Zhu et al., 2017). In prairie rabbit intestine, AQP3 is found in colon epithelium, small intestinal villus epithelium, gastric fundus; AQP4 is found in colonic epithelium, small intestine glandular epithelium, gastric fundus (Zhang et al., 2019; Zhuang et al., 2019).

AQP5: It is localized in the duodenum, on the apical membrane of secretory cells (Parvin et al., 2005). It has been indicated that AQP5 supports the development and invasion of some types of cancer (Huang et al., 2013). It has been suggested to be upregulated in several types of cancer, such as colon cancer or bile duct carcinoma (Zhu et al., 2017).

AQP6: It is expressed in the isthmus of the small and large intestine, and in the entire crypt villus axis (Laforenza et al., 2009).

AQP7: It has been detected in the gastrointestinal tract of humans, in superficial epithelial cells throughout the small intestine and colon (Zhu et al., 2017). In rats, it is thought to be necessary for rapid fluid movement in the villi epithelium, since it is found in the epithelial cells of the colon and caecum and in the apical regions of enterocytes in the villi (Lv et al., 2022).

AQP8: It is found in the colon, ileum, and jejunum, as well as in human colon villi and crypt epithelial cells (Ricanek et al., 2015; Laforenza et al., 2005).

AQP9: It has been required in goblet cell mucin synthesis and secretion, which is important in preventing intestinal infections (Okada et al., 2003). Therefore, the decreased expression of AQP9 in the ileal mucosa suggests that it may be associated with ileal mucosal damage (Xiang et al., 2018).

AQP10: It is detected in porcine jejunum, epithelial cells on the luminal villus side as well as in human ileum (Krone et al., 2019; Ansar et al., 2013).

In general, AQP1, AQP3, AQP7 and AQP11 are mostly found in the small intestine; AQP1, AQP3, AQP7 and AQP8 are plentifully expressed in the colon. Moreover, aquaporins are mostly located in intestinal villus and crypt epithelial cells. In terms of cell localization level, AQP7 and AQP10 are mostly located in the apical membrane of the cell, while AQP3 and AQP4 are in the basolateral membrane. Thus, the localization of aquaporins in the gut is compatible with their function in mediating intestinal water transport (Lv et al., 2022).

Aquaporins in intestinal diseases

Diarrhea is shaped because of disruption of water and electrolyte transport, thereby altering intestinal epithelial permeability and loss of fluid, solute, and lipid from the intestinal mucosa (Dong et al., 2020). It has been found that abnormal AQP expression, which causes diseases in the absorption and secretion of water by the gut, thus affecting intestinal membrane permeability and fluid transport also accompanies diarrhea (Engevik et al., 2018). For example, intestinal cell permeability and AQP1 expression can be inhibited by *Clostridium difficile*, leading to the occurrence and development of diarrhea (Hui et al., 2018). AQP3, AQP7 and AQP8 levels in rat colonic epithelial cells were considerably altered in the bile acid diarrhea model (Yde et al., 2016). The immunolocalization of AQP10 in the human ileum with tuberculosis disease suggests that it may cause secretory diarrhea in intestinal tuberculosis (Ansar et al., 2013). Furthermore, the abnormal decreased of aquaporin expression was showed in the antibiotic-associated rat diarrhea model (Zhang et al., 2018). Additionally, another study suggested that in the diarrhea model created by ETEC (Enterotoxigenic *Escherichia coli*), misplacement of AQP2 and AQP3 from the basolateral membrane to the infection site in the plasma membrane also occurs (Kassa et al., 2019). These data suggest that changes in the localization as well as their expression of aquaporins can alter water homeostasis in intestinal cells, thereby causing diarrhea (Lv et al., 2022).

AQP3, AQP7 and AQP8 expressions are decreased in the colon and ileum during inflammatory bowel disease (Zahn et al., 2007). In addition, downregulation of AQP1, AQP3 and AQP8 in the colon has been detected in rats with irritable bowel syndrome (IBS) (Chao and Zhang, 2018).

Constipation is a complex and common symptom of gastrointestinal diseases, and the main symptoms are dry faeces, forced defecation, and decreased defecation frequency. Normally faeces are gradually formed as a result of water absorption in colon. During this process, if the expression levels of aquaporins are high, excess water is carried from the lumen to the side of the vessel, causing hard faeces and constipation (Ikarashi et al., 2016). In various mouse constipation models, AQP2 and AQP4 expression was significantly increased in the colon (Gan et al., 2019).

Aquaporins may affect cell proliferation, migration and invasion and angiogenesis-related cell functions (Willaert et al., 2014). Therefore, there is a close relationship between cancer cell metabolism and aquaporins. Recently, aquaporins have been suggested as potential diagnostic and therapeutic targets for colorectal cancer (Kourghi et al., 2018). Various aquaporin expressions have been found to be closely associated with metastasis to lymph nodes in colon cancer in studies (Moon et al., 2003). Furthermore, prolonged downregulation of AQP8 in tumorigenesis plays an important role in terms of both potential biomarker and formation of colorectal cancer (Hong et al., 2018). In colon cancer, AQP3 has high expression levels and induce cell migration (Magouliotis et al., 2020; Moosavi and Elham, 2020). In colorectal cancer, increasing AQP5 expression is associated with late stage of lymphatic metastasis and poor prognosis (Shan et al., 2014). Aquaporins are a potential diagnostic and prognostic marker of colorectal cancer, as well as a curative target to predict treatment course (Lv et al., 2022).

Aquaporins in treatment of intestinal diseases

Today, aquaporins are frequently used as targets in the treatment of the above-mentioned diseases. There are plant extracts, drugs, probiotics, and diets used in this regard (Lv et al., 2022).

For example, it has been suggested that AQP4 expression in intestinal cells is downregulated due to the *Rotavirus* infection (Lundgren et al., 2000). Genistein, an isoflavone, can hinder *Rotavirus* replication and increase intestinal AQP4 expression. Thus, fluid absorption through intestinal cells is rearranged to manage diarrhea (Huang et al., 2015). As another example, one of the quaternary ammonium alkaloid, berberine is used as an antibiotic due to the its antidiarrheal effect by increasing water absorption due to the possible regulator effects on the expression of AQP4 in human intestinal epithelial cells (Zhang et al., 2012; Gu et al., 2011).

Several laxatives exert laxative effects by causing upregulation of AQP3 expression. Magnesium sulphate, one of the osmotic laxatives may has functions in case of osmotic pressure increase in the intestinal tract and upregulation of AQP3 expression (Zhu et al., 2017; Ikarashi et al., 2012). Bisacodyl, on the other hand, is a stimulant laxative and does not form an osmotic pressure difference, it has a laxative effect by increasing intestinal motility. It has inhibitory activity on AQP3. Because both motility is accelerated and AQP3 expression is decreased, water cannot be fully absorbed from the colon, resulting in diarrhoea (Ikarashi et al., 2011, 2012).

In a diphenoxylate-induced constipation model, therapeutic rhein decreased AQP3 expression and reduced constipation symptoms (Sun et al., 2018). However, in a loperamide-induced constipation model, the mechanism of action of loperamide was to cause inhibition of AQP3, as well as reducing intestinal motility. Naringenin, used for treatment in this model, increased the expression of AQP3 in both the basolateral and apical membranes, increased transport of water from the blood to the lumen in the direction of the osmotic gradient, and cured constipation (Yin et al., 2018).

As with osmotic and stimulant laxatives, in the case of naringenin and rhein both increase and decrease in AQP3 expression cause laxative effect. There may seem to be a contradiction here, but the reasons for these contradictions are the mechanisms that cause the disease, the mechanisms of action of the drugs used in the treatment, the positions of aquaporins in the cell membrane and the state of other aquaporins at that time.

Besides the drugs mentioned above, probiotic products and some diets may also enhance intestinal water transport function by regulating aquaporins. Non-enterotoxigenic *Bacteroides fragilis* (NTBF) is considered a gut-protecting probiotic and has been used to treat diarrhoea by increasing the expression of AQP3 and AQP8 (Zhang et al., 2018). In another study, a probiotic mixture (consisting of *Lactobacillus acidophilus* DM8302, *Bifidobacterium breve* DM8310 and *Lactobacillus casei* DM8121, mixed in a 1:1:1 ratio and grown as a bacterial mass) can regulate AQP3 expression to augment the water content of the faeces and ultimately enhance constipation (Deng et al., 2018).

As well as probiotics, diets containing trihexanoin (Wu et al., 2018), alpha ketoglutaric acid (He et al., 2017), functional complex amino acids (Yi et al., 2018), low protein, and high carbohydrate (Fan et al., 2017) are also used in the treatment of diarrhoea by increasing the expression of various aquaporins.

Conclusion

The widespread expression of aquaporins in the intestinal tract of mammals suggests that these channels are closely related to the normal physiological function and health of the intestines. When the pathophysiology of intestinal diseases is examined, it is seen that abnormal expression and localization of aquaporins in intestinal epithelial cells may play a role in the pathogenesis of diseases. Therefore, regulation of aquaporins by drugs and/or dietary supplements to improve intestinal physiological state is of great importance in preventing or treating intestinal diseases. However, the way to regulate aquaporin gene expression is still controversial. Therefore, further studies are needed to develop more specific drugs and/or dietary supplements for aquaporins and their regulation, as well as to elucidate the role of aquaporins as a marker of intestinal health status and a target of intestinal health-regulating agents.

Acknowledgements

This review article was prepared from the first doctoral seminar of Miray AYKOÇ.

Conflict of Interest

No conflict of interest was declared by the authors.

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Animal Health Prod and Hyg (2022) 11 (2) : 56 - 65

Animal Health, Production and Hygiene

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Review

Bovine *Escherichia coli* Mastitis and Effects on Milk Microbiota

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A B S T R A C T

Escherichia coli is a microorganism that is found in the normal intestinal microbiota of humans and warm-blooded animals, causing intestinal or extra-intestinal infections. Many pathogenic *E. coli* strains can cause diarrhea, septicemia, neonatal meningitis, mastitis, urogenital system infections and various intra-abdominal, lung, soft tissue and skin infections in pets. Determining the genotypic and phenotypic characteristics of *E. coli* strains isolated from animals is very important for the prevention of infections caused by *E. coli*. In recent years, a new animal pathotype mammary pathogenic *E. coli* (MPEC), which causes mammary gland infections in animals has been included in the extraintestinal pathogenic *E. coli* group. The fact that approximately 25%-35% of the use of antimicrobials in the treatment of Gram-negative agents is unsuccessful indicates that the use of correct diagnostic tools should take place in routine before etiological diagnosis. Profiles in bovine milk with mastitis suggest that clinical mastitis is associated with dysbacteriosis and that the microbial community in an intact mammary gland helps prevent intramammary infection. In this review, the change in bacterial diversity of milk microbiota due to antimicrobial use in *E. coli*-induced mastitis cases is discussed together with current studies.

***Keywords**: Escherichia coli, mastitis, microbiota*

Sığır *Escherichia coli* Mastitisleri ve Süt Mikrobiyotası Üzerine Etkileri

Ö Z E T

Escherichia coli, insan ve sıcak-kanlı hayvanların normal barsak mikrobiyotasında yer alan ve aynı zamanda intestinal veya ekstra-intestinal infeksiyonlara yol açan bir mikroorganizmadır. Birçok patojenik *E. coli* suşları, evcil hayvanlarda diyare, septisemi, neonatal meningitis, mastitis, ürogenital sistem infeksiyonları ile çeşitli intra-abdominal, akciğer, yumuşak doku ve deri infeksiyonlarına neden olabilmektedirler. Hayvanlardan izole edilen *E. coli* suşlarının genotipik ve fenotipik özelliklerinin belirlenmesi, *E. coli*'nin neden olduğu infeksiyonların önlenmesi açısından oldukça önemlidir. Son yıllarda, ekstraintestinal patojenik *E. coli* grubuna yeni bir hayvansal patotip olan ve hayvanlarda meme bezi enfeksiyonlarına yol açan meme patojenik *E. coli* (MPEC) dahil edilmiştir. Gram negatif etkenlerin tedavisinde antimikrobiyallerin kullanımının yaklaşık %25-%35'inin olumsuz sonuçlanması doğru tanı araçlarının kullanımının etiyolojik tanıdan önce rutinde yerini alması gerektiğini göstermektedir. Mastitisli sığır sütündeki profiller, klinik mastitisin disbakteriyoz ile ilişkili olduğunu ve sağlam bir meme bezindeki mikrobiyal topluluğun meme içi enfeksiyonu önlemeye yardımcı olduğunu göstermektedir. Bu derlemede *E. coli* kaynaklı mastitis olgularında antimikrobiyal kullanımına bağlı olarak süt mikrobiyotasının bakteri çeşitliliğindeki değişim güncel çalışmalarla birlikte ele alınmıştır.

***Anahtar kelimeler**: Escherichia coli, mastitis, mikrobiyota*

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Received Date: 25.09.2022 - Accepted Date: 21.10.2022

DOI: 10.53913/aduveterinary.1179963

Introduction

Escherichia coli (E. coli) was isolated from infant feces in 1885 by the German microbiologist and pediatrician Theodor Escherich and named as *Bacterium coli commune* In 1895, with the revision of the Bacterium genus, it was reclassified by Migula as *Bacillus coli*. Finally, in 1919, it was revised as *Escherichia coli* in honor of Theodor Escherich (Blount, 2015). As a result of the increase in the amount of *E. coli* in the intestinal microbiota of humans and animals, various infections may ocur (İzgür et al., 2006). Pathogenic *E.coli* strains are zoonotic. It can be transmitted to humans through direct contact with the feces of animals or consumption of food and water contaminated with feces (Müştak et al., 2013). The factors that play a role in the emergence of *E. coli* infections can be listed as inadequate hygienic conditions, not giving colostrum to newborn puppies, not vaccinating, increase in the number of intestinal microbiota, nutritional disorders and stress (İzgür et al., 2006).

E. coli general characteristics, typing and pathotypes

E. coli is a Gram-negative, generally motile, cylindrical, non-spore-shaped, bacillus bacterium belonging to the *Enterobacteriaceae* family. Some strains of *E. coli* contain capsules and fimbriae (Gomes et al., 2016). Escherichia genus is located in the *Enterobacteriaceae* family in the *Gammaproteobacteria* class, which is the largest subclass of *Proteobacteria* (Brenner et al., 2005). *E. coli* replicates approximately every 20 minutes. It can grow rapidly in aerobic or anaerobic environment in a wide temperature range of 18 °C - 44 °C in liquid and general solid media (Jang et al., 2017). Colonies of *E. coli* strains formed on blood agar and nutrient agar are convex, shiny, smooth-edged and colorless. Due to lactose fermentation on MacConkey agar, it produces bright, pink colonies surrounded by a precipitate. On EMB agar, they form dark, green-black metallic green colonies (Prahlad et al., 2018). *E. coli* is an oxidase negative, catalase positive, nitrate reduction positive and mostly lactose fermenting bacteria (Al Humam, 2016). Commercial kits such as API 20E, which include customized tests for the determination of biochemical properties, can be used to distinguish *E. coli* from bacteria in the *Enterobacteriaceae* family and other *Escherichia* species (Liu et al., 2015).

Guanin sitozin percentage (G+C) content of *E. coli* strains is between 48 and 59. The average DNA relationship calculated based on DNA-DNA hybridization (DDH) varies between 29% and 94% within *Escherichia* species, and the DNAs of different *E. coli* strains are closely related with an average of 84% (Scheutz and Strockbine, 2015).

E. coli strains are subtyping using the combination of the three main surface antigens, somatic O, flagellar H and capsular K antigens (O:K:H), and serotyping based on O and H antigens is considered the gold standard, since the possibility of K antigen typing is limited (DebRoy et al., 2011).

Phage typing reveals the pathogenic roles of serologically unidentified *E. coli* strains. Due to the production difficulties of antisera, phage typing of *E. coli* K1, K3, K5, K7, K12, K13 and K95 strains is very useful for some antigens (Scheutz and Strockbine, 2015).

Clermont et al. (2000) in 2000, they defined the triplex PCR method called Clermont Typing by providing a simple and rapid detection of *E. coli* phylogroups based on the detection of *E. coli* haem-utilization (chuA) gene, uncharacterized protein yjaA gene and tail - specific protease (TSPE4.C2). Later, in the light of new genomic data, the arpA gene was added to the protocol as an additional gene region, and the triplex PCR method was revised as the quadruplex PCR method (Clermont et al., 2013). In recent years, with the evaluation of whole genome sequence data, some *E. coli* strains have been included in a new phylogroup defined as phylogroup G between phylogroup B2 and phylogroup F (Clermont et al., 2019).

Virulence genes are not the only feature used to distinguish pathogenic *E. coli* strains from commensal strains, but phenotypic determination of virulence genes is also an important factor. *E. coli* strains are divided into two as those that cause intestinal (intestinal) and extra-intestinal (extraintestinal) infections (Omerovic et al., 2017). *E. coli* strains isolated from intestinal diseases were divided into different main categories according to phenotypic features, epidemiological features, clinical features of the diseases they cause, and specific virulence factors. Intestinal *E. coli* pathotypes; It consists of Enterotoxigenic *E. coli* (ETEC), Enteroaggregative *E. coli* (EAEC), Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), Diffuz Aderent *E. coli* (DAEC) and Vero- or Shiga-toxin producing *E. coli* (VTEC or STEC) (Scheutz and Strockbine, 2015). Extraintestinal pathogenic *E. coli* (ExPEC) strains are facultative pathogens and are commensal in the intestinal microbiota of healthy animals. Pathotypes in the ExPEC group; It includes Septicemic Pathogenic *E. coli* (SEPEC), Neonatal Meningitis *E. coli* (NMEC), Uropathogenic *E. coli* (UPEC), Avian Pathogenic *E. coli* (APEC) and other potential *E. coli* pathotypes. In recent years, mammary pathogenic *E. coli* (MPEC), which causes mammary gland infections in animals, and endometrial pathogenic *E. coli* (EnPEC), which causes uterine infections, have been added to other potential *E. coli* pathotypes (Omerovic et al., 2017).

Mammary pathogenic E. coli (MPEC)

Apart from the typical factors involved in *E. coli* virulence in general, the specific pathogenic mechanisms specific to MPEC strains are not yet clearly known. It is stated that mastitis occurs after local immune response triggered after detection of various *E. coli* compounds by mammary gland epithelium and immune cells. The most known of these compounds is lipopolysaccharide, which is considered to be the major virulence factor in mastitis caused by *E. coli*. In studies conducted on *E. coli* mastitis, MPEC strains were determined to cause acute (VL2874 and P4 strains) or chronic (VL2732 strain) mas-

Table 1. Characteristics of MPEC strains (Blum et al., 2015).

Strain	Source	Serotype	Phylogenetic group	Development in milk/ Phagocytosis resistance	Mammary pathogenic Mouse/Cow
VL2874	Acute masititis	O141:H4	A	Fast/High	Yes/Yes
VL2732	Chronic mastitis	O8:H30	A	Fast/High	Yes/Yes
P4	Acute masititis	O32:H37	A	Fast/High	Yes/Yes
K71	Barn	O58:H40	B1	Slow/Low	No/No

titis and shown in Table 1 (Blum et al., 2015).

E. coli VL2874 strain has the RTX toxin cluster containing the hemolysin genes hlyA, hylC, and hylD. VL2874 is the only strain that shows hemolytic character in sheep blood agar among other mastitis agent MPEC strains. hylA has been found to increase pathogenicity in ExPEC strains. In addition, TosA, which is very similar to the RTX toxin, has been associated with pathogenicity in UPEC strains (Vigil et al., 2011). In the search for the *E. coli* plasmids database in NCBI, it was determined that Col (RNAI) identified in the genome of the VL2874 strain, is a plasmid type that is very closely related to the ColE-1-like plasmid p302S (AY333433) found in *Salmonella enterica subsp. enterica*. VL2732 strains contain the toxin mRNA interferase YgiU or MqsR, which transforms into permanent cells under biofilm formation and stress, and the yersiniabactin siderophore, which is also associated with biofilm formation in iron-limited environments such as milk. Yersiniabactin, which is also found in APEC strains, plays an important role in the formation of chronic mastitis (Blum et al., 2015).

Effects of E. coli on microbiota in clinical mastitis

A 100-year review of the Journal of Dairy Science states that mastitis, defined as inflammation or inflammation of the mammary gland, remains the most common bacterial disease in dairy farms. In addition, it is stated that studies continue on the treatment of mastitis or preventing the use of frequently used antibacterials. In clinical mastitis cases, a treatment protocol is established depending on the results of aerobic culture, and the treatment of Gram-positive mastitis agents is generally successful. Studies on pathogen-based protocols later showed that the efficacy of intramammary antimicrobial applications against Gram-negative mastitis agents may be insufficient (Vasquez et al., 2019). The fact that approximately 25%-35% of the use of antimicrobials in the treatment of Gram-negative agents is unsuccessful indicates that the use of correct diagnostic tools should take place in routine before etiological diagnosis (Oliveira and Ruegg, 2014). Sequencing and analysis of highly variable regions in the 16S rRNA gene can assess bacterial diversity in the milk of cows with mastitis, as well as reveal which organisms contribute to culture-negative and other etiological diseases (Oikonomou et al., 2014).

Porcellato et al. (2020) described that milk in healthy cattle has a complex microbiota, mainly composed of *Corynebacteriaceae* and *Staphylococcaceae* families. They identified these two families in all cows and therefore identified them as part of the core microbiota with potential implications for udder health. Profiles formed in bovine milk with mastitis suggest that clinical mastitis is associated with dysbacteriosis and that the microbial community in an intact mammary gland helps prevent intramammary infection (Fernández et al., 2013; Oikonomou et al., 2014).

Ganda et al. (2017) reported high microbial diversity in the microbiota before the experimental infusion of *E. coli* into the breast (72 hours before). It was determined that there was no difference between the microbial profile in the 4 groups formed before the study. In the samples of all groups before the study, the most abundant families were found as, *Ruminococcaceae* (16.8%±10.1%), *Lachnospiraceae* (7.0%±5.1%), *Aerococcaceae* (6.8%±8.2%), *Enterobacteriaceae* (6.3%±13.5%), *Planococcaceae* (5.7%±9.5%), *Bacteroidaceae* (5.4%±3.3%), *Corynebacteriaceae* (5.1%±7.3%), *Clostridiaceae* (4.2%±3.1%), *Bacillaceae* (3.5%±3.7%) ve *Staphylococcaceae* (2.8%±4.9%) in order from the most dense to the least dense. It is seen that the microbial diversity is much higher in the unchallenged groups than in those with experimental mastitis infection with *E. coli* (Figure 1a). It was determined that there was no significant change in the mean densities of the 25 families, which were determined to be the most intense before the study, in the group in which intramammary ceftiofur was administered without any experimental infection (Figure 1b). In the challenged untreated group after the challenge application, it remained above the 30% average intensity between 12 and 150 hours and reached a peak level of 64.7% at the 12th hour (Figure 1c). In the challenged treated group after the challenge application, it remained above the 30% average intensity between 12 and 600 hours and reached a peak level of 77.9%

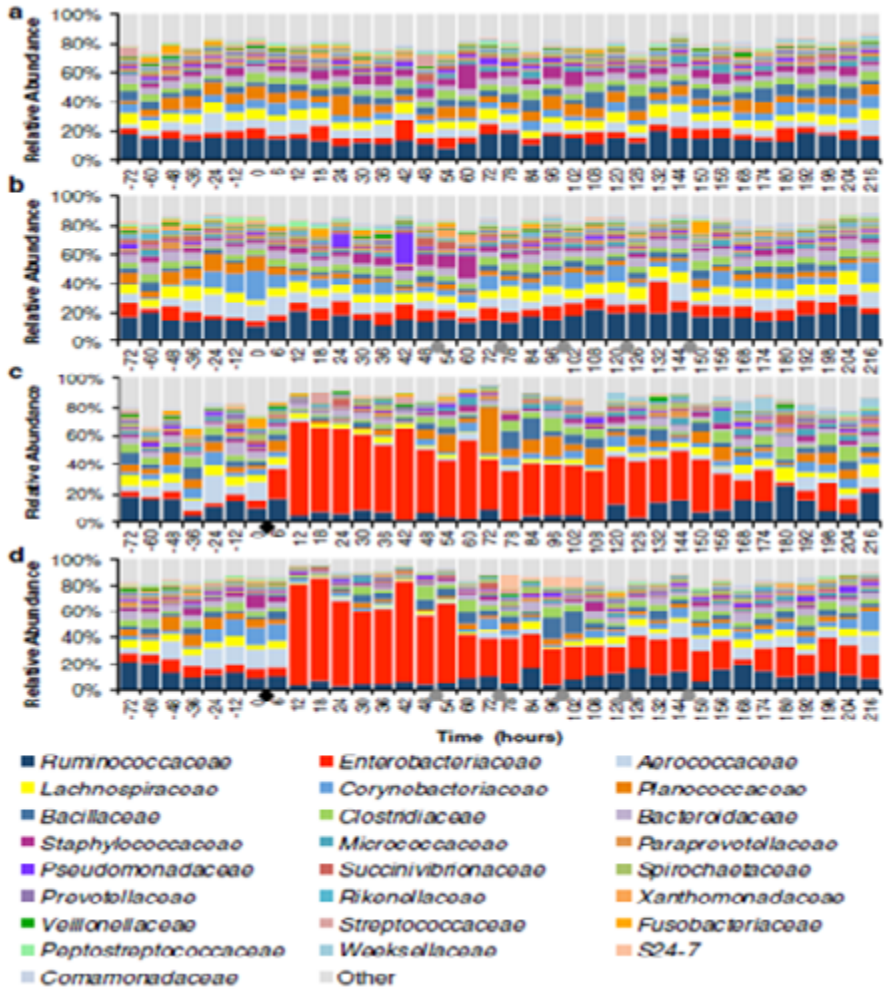

Figure 1. The effect of ceftiofur treatment and *E. coli* challenge application on the average densities of 25 families in milk microbiota in cattle (Ganda et al., 2017).
a. Unchallenged and untreated group
b. Unchallenged and treated group,
c. Challenged and untreated group,
d. Challenged and treated group.
(Black backpoints represent experimental infection with 100 CFU of *E. coli* and grey backpoiints represent intramammary treatment with ceftiofur)

at the 18th hour (Figure 1d). It was observed that the microbial profile of the milk changed significantly in the groups challenged with *E. coli* (Figure 1c, 1d).

Before the challenge was applied, *Ruminococcaceae* was determined as the most dense family. The mean densities of the *Ruminococcaceae* family were found to be 14.3% and 13.3% respectively in the challenged treated and challenged untreated groups. A significant increase in the densities of the *Enterobacteriaceae* family was detected in the challenge applied groups (Figure 2). Intramammary administration with ceftiofur hydrochloride did not show a significant effect on the average density ratio of the *Enterobacteriaceae* family, and it also did not provide a significant decrease in any time zone after the challenge test when compared to the untreated group (Figure 2).

They stated that Shannon diversity index values were high and similar in all groups before the challenge application. Diversity values decreased sharply after *E. coli* challenge application and the lowest diversity values were observed 30 and 78 hours after challenge application. They found that challenged treated animals had significantly different indices of diversity than challenged untreated animals at 78, 102 and 180 hours. They did not detect any significant difference between the diversity levels of the unchallenged treated and unchallenged untreated groups (Figure 3).

Ganda et al. (2017) found that 3 of 12 cows that were challenged in the study were not infected. Schukken et al. (2009) and Burvenich et al. (2003) stated that the high number of somatic cells in the time period before the challenge was stated as the best explanation for this. Animals infected after challenge administration showed a sharp increase in linear score 18 hours after administration. They found that linear scores were higher in animals that were challenged but not infected, compared to those that were infected before the challenge (Figure 4).

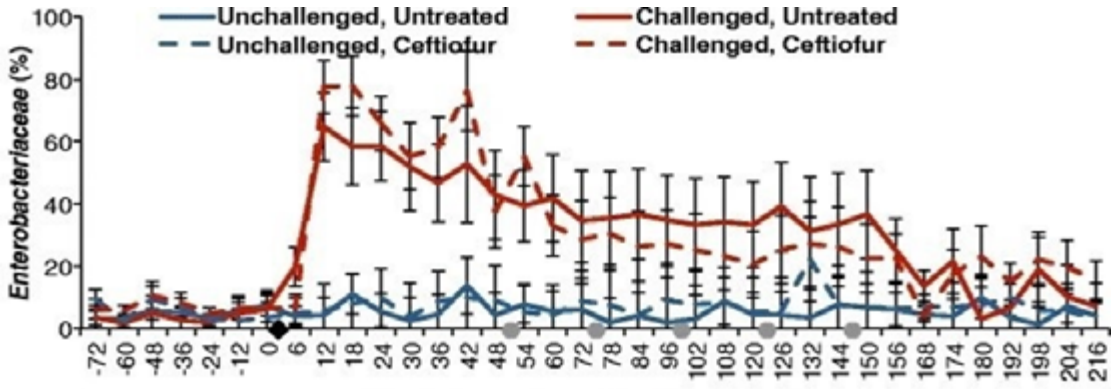

Figure 2. Effects of experimental infection with *E. coli* and treatment with ceftiofur on average densities of the *Enterobacteriaceae* family (Ganda et al., 2017).
(Black backpoints represent experimental infection with 100 CFU of *E. coli* and grey backpoints represent intramammary treatment with ceftiofur)

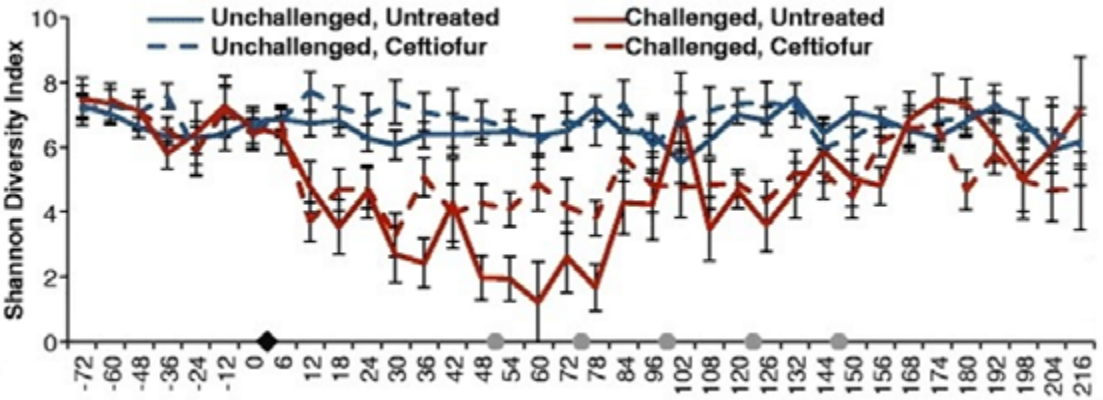

Figure 3. Shannon diversity index graph of microbiota as a result of experimental infection with *E. coli* and treatment with ceftiofur (Ganda et al., 2017).
(Black backpoint represent experimental infection with 100 CFU of *E. coli* and grey backpoints represent intramammary treatment with ceftiofur)

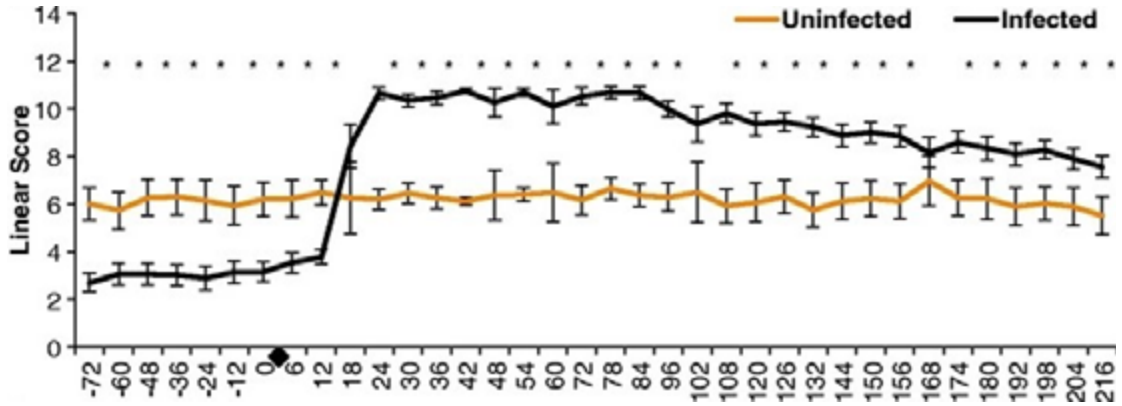

Figure 4. Effects of experimental infection with *E. coli* on linear score values in infected and uninfected cows (Ganda et al., 2017). (Black backpoint represent experimental infection with 100 CFU of *E. coli*)

As a result of the study, Ganda et al. (2017) determined that the application of ceftiofur treatment in *E. coli*-induced mastitis did not cause a significant change in the milk microbiome.

Lima et al. (2018) determined that *Firmicutes* (57.7%±7.6%) and *Proteobacteria* (26.0%±7.6%) phyla predominate as a result of sequence analysis performed on healthy milk samples. When milk sample fractions (whole milk, fat, oil+pellet, pellet) and extraction kits (PowerFood, PowerSoil) were compared, they found that there was no significant difference in the mean values of these two phyla. They reported that the *Proteobacteria* phylum in *E. coli* and *Klebsiella spp.* mastitis samples comprised approximately 98% of the 16S rRNA sequences detected independently of milk fractions and DNA extraction kits. In milk samples with mastitis caused by *Streptococcus spp.* most of the sequences were

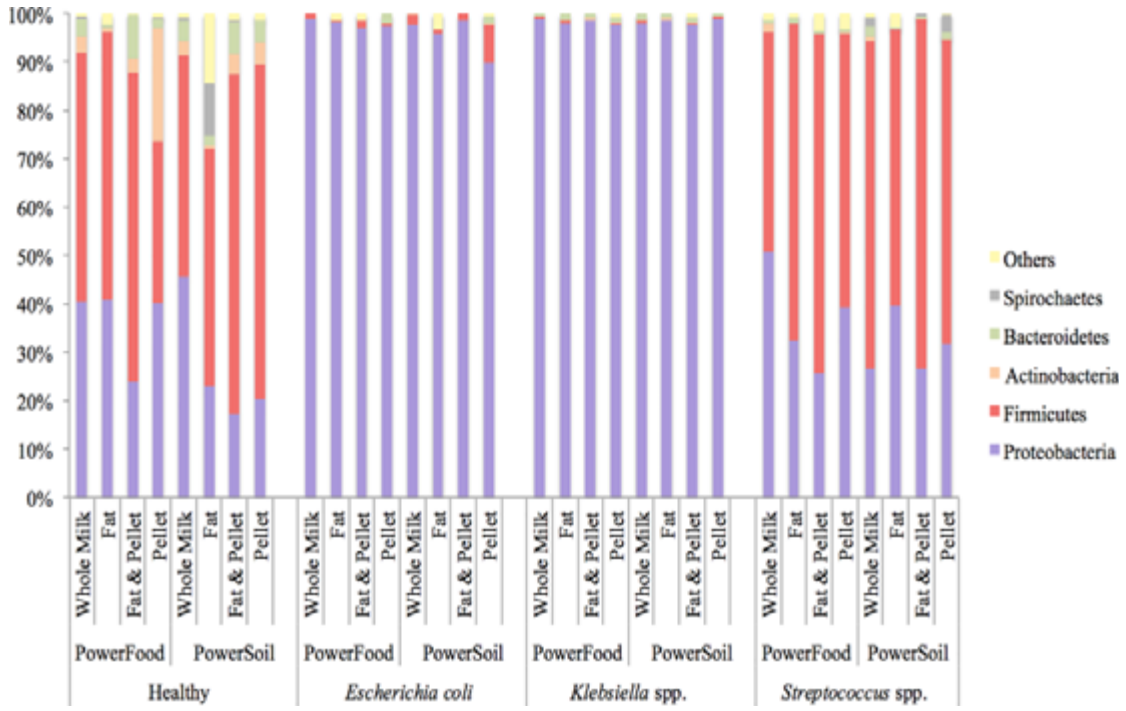

Figure 5. Mean relative abundance of the most prevalent bacterial phyla identified in healthy milk samples and milk samples from cows diagnosed with clinical mastitis due to Escherichia coli, Klebsiella spp. and Streptococcus spp. infection according to four milk sample fractions (whole milk, fat, fat + pellet, and pellet) and two different DNA extraction kits (PowerFood and PowerSoil) (Lima et al., 2018).

found to be associated with *Firmicutes* (69.6%±9.5%) and *Proteobacteria* (30.1%±9.4%) (Figure 5).

They stated that they detected 62 families in common in two DNA extraction kits in healthy milk samples on the Venn diagram (Figure 6a). It has been determined that these 62 families constitute 95.65% of the average density in healthy milk samples. *Ruminococcaceae*, *Enterobacteriaceae*, *Staphylococcaceae*, *Bacillaceae*, *Streptococcaceae* and *Pseudomonadaceae* were the most common bacterial families detected in all fractions of healthy milk samples (Figure 6b). On the venn diagram created according to the sample fractions, it was stated that 4 families within 28 common families constitute the main microbiota (87.8%±2.4%) defined among all milk sample fractions (Figure 6c). They determined that the four families that make up this main microbiota consist of *Ruminococcaceae*, *Enterobacteriaceae*, *Bacillaceae* and *Pseudomonadaceae*.

Lima et al. (2018) identified 4 families as common by two extraction kits among 30 families detected on the venn diagram in milk with mastitis originating from *E. coli* (Figure 7).

It has been revealed that the determination of the *Enterobactericeae* family in the isolations made in the two DNA isolation kits was done correctly and there was no significant difference between the average densities determined in the protocols. It was determined that only in the pellet sample fraction, the PowerFood extraction kit found the average density of the *Enterobacteriaceae* family 10% higher than that of PowerSoil (Figure 8).

As a result of the study by Lima et al. (2018) the total number of families determined as a result of sequencing in *E. coli* and *Klebsiella spp.* mastitis samples were 30 and 28, respectively; It was determined as 64 in mastitis samples originating from *Streptococcus spp.* The results of this study show that the milk microbiome changes very significantly in cases of mastitis originating from the *Enterobacteriaceae* family, especially *E. coli* and *Klebsiella spp*.

Ganda et al. (2016) in metagonomic analyzes of milk microbiota, showed that the mean density of *Proteobacteria* phylum was higher in milk with mastitis caused by *E. coli*, *Klebisella spp.* and *Pseudomonas spp.* compared to healthy milk. In the comparison made with the causative agent, the rise of the phylum it belongs to during the infection was mostly seen in the milk with mastitis caused by *E. coli* (Figure 9).

Ganda et al. (2016) applied ceftiofur to animals with *E. coli* mastitis for 5 days in their study. They stated that the general bacterial load decreased on the 3rd day in the treated group compared to the untreated group, but no effect was observed at the end of the 8th day. As a result of the study, the control group (infected and non-infected), treated and untreated groups were thoroughly examined. At the end of the 14th day, they determined that the bacterial diversity was higher in the milk samples of the treated and untreated milk samples of those who did not show clinical signs. The mean relative abundance of bacteria from the phylum *Proteobacteria* was greater in the milk from mastitic quarters infected by *E. coli* and *Pseudomonas spp.* compared

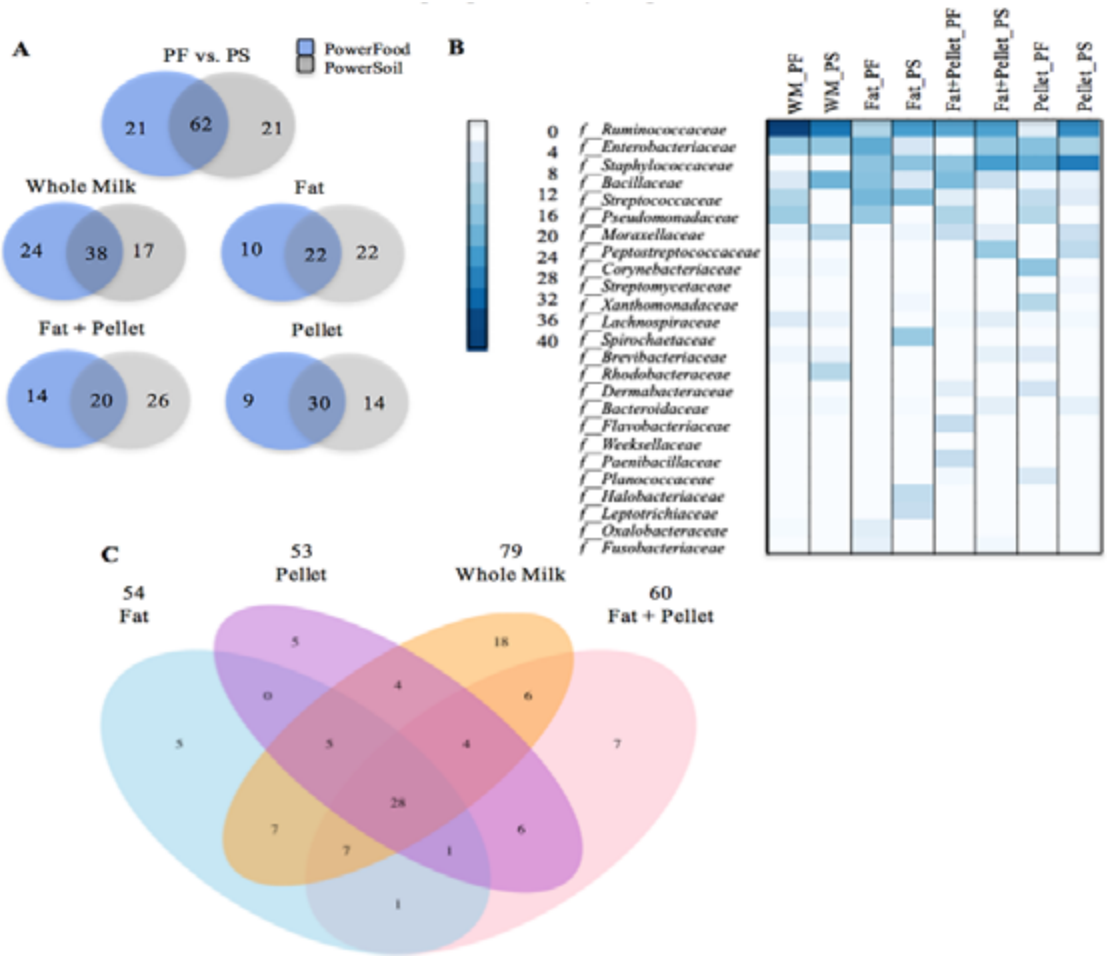

Figure 6. The most common bacterial families detected by Venn diagram showing the degree of overlap of bacterial families between two DNA extraction kits in 4 sample fractions in healthy milk samples (Lima et al., 2018).
a. Venn diagrams showing the numbers of unique and shared bacterial families for healthy milk samples,
b. Heatmap illustrating the 25 most common bacterial families ranking by relative abundance identified in healthy milk samples according to milk sample fractions: fat, fat + pellet, pellet, and whole milk and DNA extraction kit,
c. Venn diagram showing the numbers of unique and shared bacterial operational taxonomic unit according to milk sample fractions. Numbers at the top of each milk sample type are the total number of families detected in samples processed by that protocol.

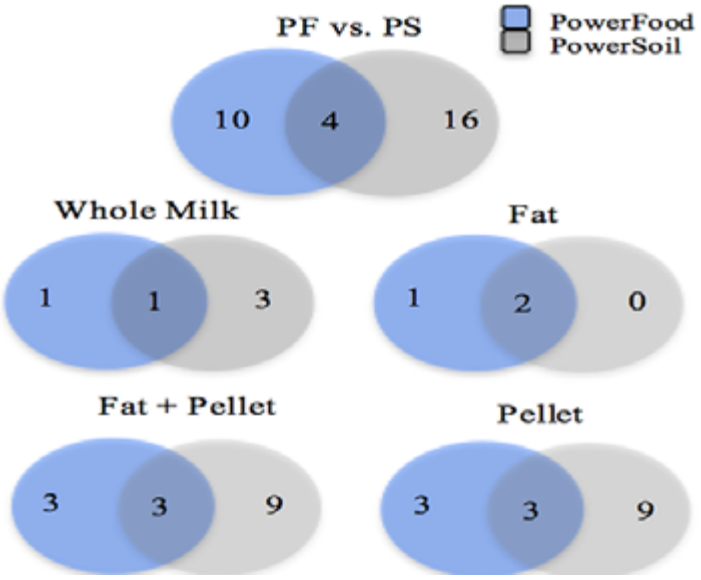

Figure 7. Venn diagrams showing the degree of overlap of bacterial families between two DNA extraction kits relative to sample fractions of milk with E. coli-induced mastitis (Lima et al., 2018).

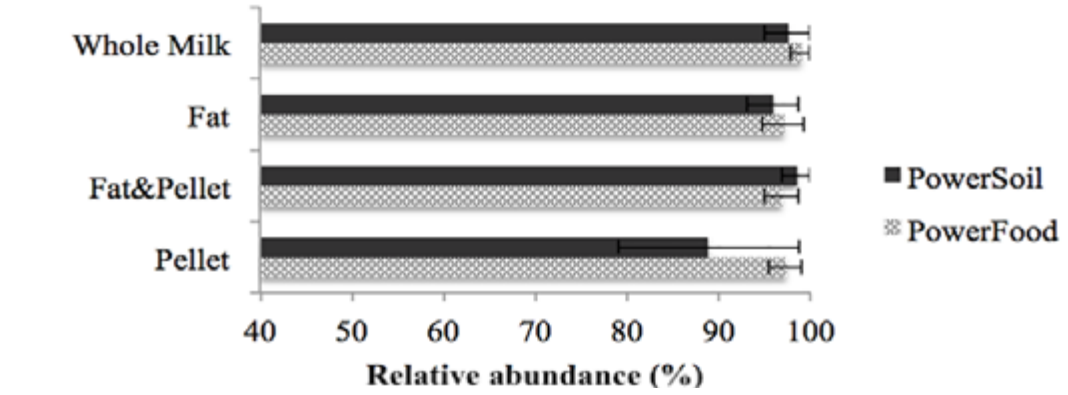

Figure 8. Average densities of the Enterobacteriaceae family detected in each milk sample fraction (Lima et al., 2018).

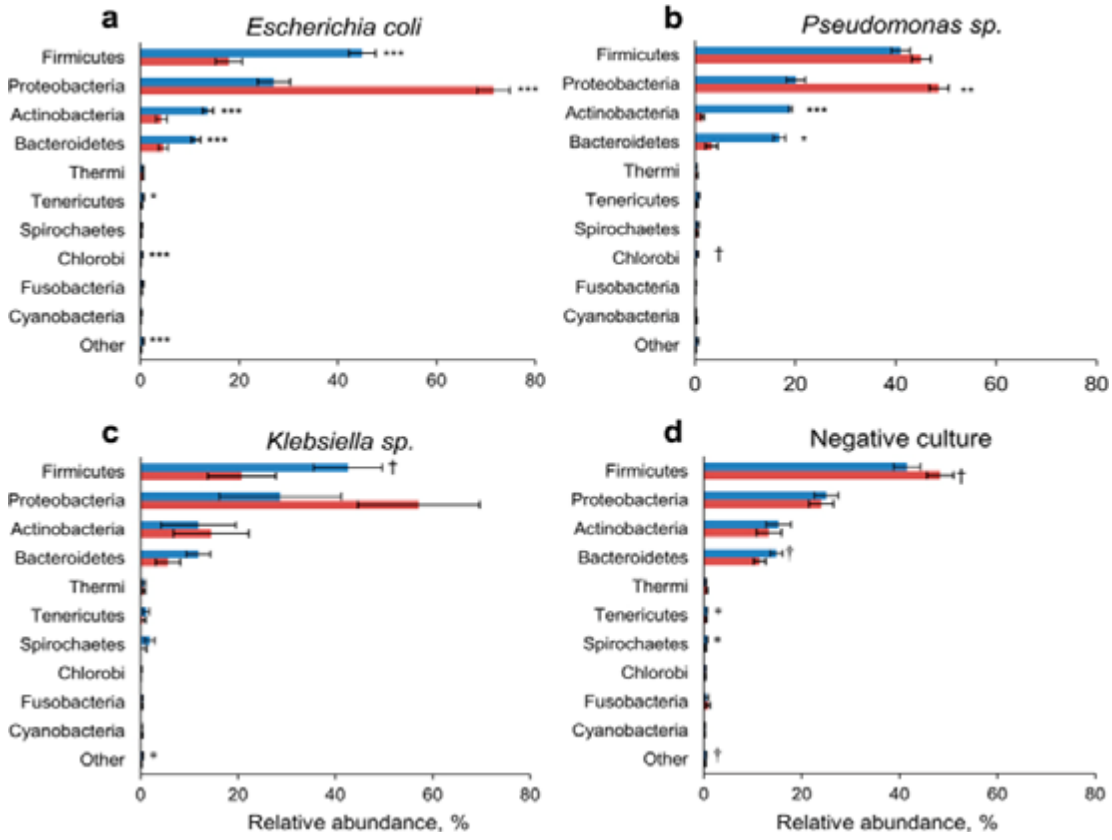

Figure 9. Average phylum densities in milk with clinical mastitis of different origin (red bars) and healthy milk (blue bars) (Ganda et al., 2016).

with that of healthy quarters. This was driven mostly by greater abundances of *Enterobacteriaceae* (Figure 10).

Conclusion

In dairy farming the inability to make a correct diagnosis against mastitis infections, the use of wrong antibiotics, the increase in antibiotic resistant bacterial populations caused by antibiotic use, and the decrease in bacterial diversity in milk microbiota are stated as the most basic problems today. The use of antimicrobials in the food industry is considered as one of the potential factors that may affect human health apart from its effective use. Ceftiofur is the only third generation cephalosporin FDA approved for use in food production animals, especially against gram-negative agents and *Enterobacteriaceae* family infections such as *E. coli*. However, it has been classified as one of the critically important antimicrobials for humans by the World Health Organization. Studies on milk microbiota have also shown that the efficacy of ceftiofur treatment is not sufficient in cases of culture-negative, *E. coli* and other causative mastitis. Studies have shown that bacterial diversity in milk microbiota decreases more significantly in *Enterobacteriaceae* and especially in *E. coli*-induced mastitis cases compared to other causative mastitis cases. It is necessary to characterize dysbacteriosis cases on the basis of the agent in mastitis cases, and to consider the bacterial diversity in the microbiota, treatment protocols, linear score and changes in milk yield after infection. In addition to these factors, it has been concluded that long-

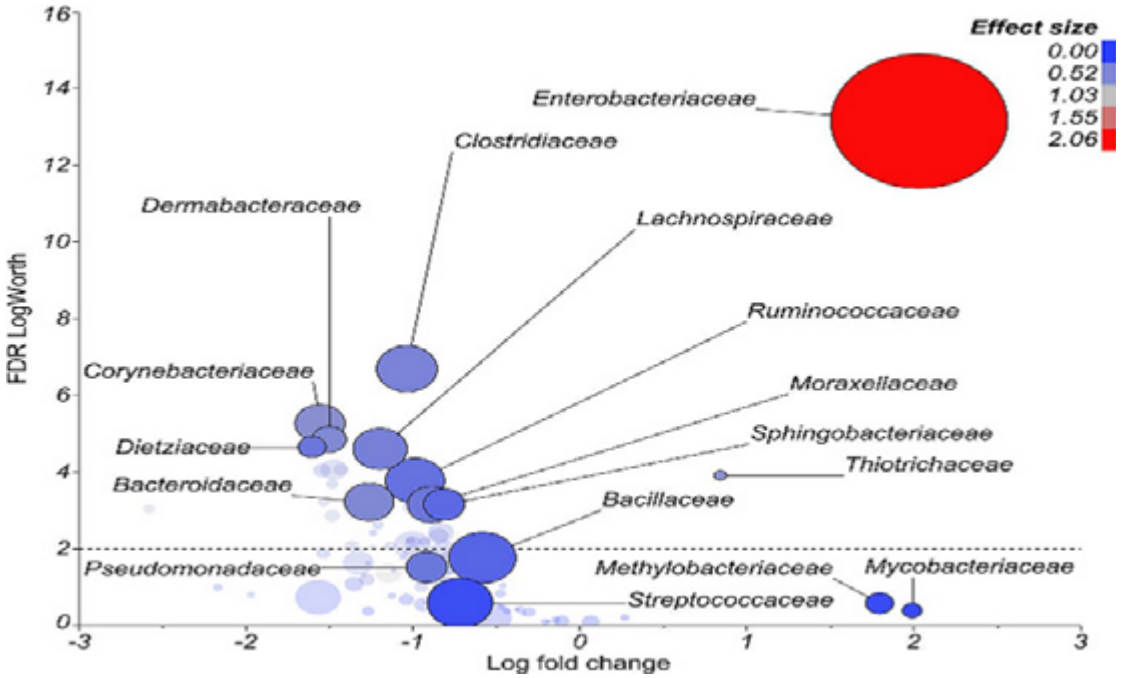

Figure 10. Comparison of the microbiome from quarters with clinical mastitis associated with E. coli and healthy quarters (Ganda et al., 2016).

term and detailed evaluations are required in scientific studies on the milk microbiome, taking into account the clinical condition of the animals.

Acknowledgements

This review article was prepared from the second doctoral seminar of the corresponding author.

Conflict of Interest

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

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Correction

Morphometric Evaluation of the Relationship Between the Distal Femur and Proximal Tibia of the Dogs

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ABSTRACT

In this study, 42 adult dogs, comprised of 25 males and 17 females, with 18 different morphological types from the heterogeneous population were used. A total of 4 osteometric measurements of the femur and tibia were taken. After calculating the mean and standard deviation values of 42 adult dogs (SPSS 21.0 program), the T-test was applied to observe the significance of the difference between the mean values of both sexes. Correlations were observed between osteometric measurements of the femur and tibia. As a result, regression formulations were created to make independent estimations of both the femur "greatest breadth of the distal end" and tibia "greatest breadth of the proximal end". The formulations formed had a high specificity value (R2). Hence, while sexual dimorphism was not observed on the measurements, the data produced in this study will contribute to the knee joint operations as well as to the identification of the same individual in mass burial contexts in zooarchaeological studies.

***Keywords**: Correlation, dog, femur, morphometry, tibia*

Köpeklerin Distal Femur ve Proksimal Tibiası Arasındaki İlişkinin Morfometrik Değerlendirimesi

ÖZET

Bu çalışmada heterojen popülasyondan 18 farklı morfolojik tipe sahip 25 erkek ve 17 dişi olmak üzere 42 yetişkin köpek kullanıldı. Femur ve tibiadan toplam 4 osteometrik ölçüm alındı. Ortalama ve standart sapma değerleri hesaplandıktan sonra, her iki cinsiyetin ortalama değerleri arasındaki farkın anlamlılığını gözlemlemek için T testi uygulandı. Femur ve tibianın osteometrik ölçümleri arasında korelasyonlar gözlendi. Tibia'nın proximal'i ve femur'un distal'inde maximum genişlik ölçümleri hakkında bağımsız tahminler yapmak için regresyon formülasyonları oluşturuldu. Oluşturulan formülasyonlar yüksek bir özgüllük değerine (R2) sahipti. Dolayısıyla, ölçümlerde cinsel dimorfizm görülmezken, bu çalışmada ele edilen veriler diz eklemi operasyonlarına ve zooarkeolojik çalışmalarda toplu gömülme durumlarında aynı bireyin tanımlanmasında katkı sağlayacaktır.

***Anahtar Kelimeler**: Korelasyon, köpek, femur, morfometri, tibia*

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Received Date: 16.04.2022 – Accepted Date: 23.05.2022

DOI: 10.53913/aduveterinary.1104696

Introduction

Dogs have different sizes and visual morphologies, and skull typology has been widely used to distinguish and define morphological types (Stockard, 1941; Komeyli, 1984; Brehm et al., 1985; Regedon et al., 1991; Onar, 1999; Onar et al., 2001). With this, long bone morphometry was used as the most basic element in determining the visual morphological characteristics such as body weight and shoulder height of different types of dogs and equations were created (Harcourt, 1974; Anderson et al., 1985; Anyonge, 1993; Wroe et al., 1999; Onar, 2005; Onar and Belli, 2005). Another important factor affecting visual morphology is sexual dimorphism, which reveals the size difference between males and females (Nganvongpanit et al., 2017). The fact that dogs from Yorkshire terrier to Dobermann have different sizes and morphological structures has brought with it intraspecific polymorphism (Jouve et al., 2001). Therefore, most of the common orthopaedic diseases observed in dogs are due to physical conformation and genetic predisposition (Boge et al., 2019). It was argued that many of the orthopaedic problems of long bones occur in the femur and its joints, and the conformation and geometry of the femur in dogs was emphasized (Ocal et al., 2012; Kara et al., 2018). The tibia, located distal to the femur, has also been evaluated for many races, both anatomically and angularly (Sabancı and Ocal, 2014, 2016; Aertsens et al., 2015). The knee joint, in which the femur and tibia participate, is a complex joint both morphologically and functionally (De Rooster et al., 2006; Gupte et al., 2007). This joint is composed of articulatio femorotibialis, articulatio femoropatellaris and articulatio tibofibularis proximalis and includes not only the femur and tibia as its components, but also the poximal parts of the patella and fibula (Dyce et al., 1987; Evans 1993; Bahadır and Yıldız, 2010). Articulatio femorotibialis, which is shaped between the condyles of the femur and tibia, is a ginglymus-like joint and carries the meniscus, which eliminates the compatibility between the rounded femur bone and the flat tibia bone plateau (Bahadır and Yıldız, 2010). The joint is also supported by distinctive cruciate ligaments (Dyce et al., 1987; Evans 1993). With this structural feature, this joint has been examined by many researchers in terms of both morphometric and angular aspects of the bones involved in its formation, and data that will contribute to clinical evaluations have been revealed (Ocal et al., 2012; Sabancı and Ocal, 2014, 2016; Kara et al., 2018).

In this study, using distal femur and proximal tibia morphometries, the relationship between these discordant parts of both bones was evaluated. Except for the angular measurements of both bones, the exchange of morphometric data with each other has been documented. Orthopedically speaking, this joint is a joint prone to injuries such as patella luxation, cruciate ligament ruptures and meniscus deformations due to its structural feature in dogs (Singleton, 1963; Barrett et al., 2009), it is of particular importance to know the relationship between the femur distal and tibia proximal extremity or end. In line with the morphometric data obtained, it was aimed to create the best regression equation to be used in possible prosthetic applications (Altunatmaz et al., 2019).

Material and Method

A total of 42 adult dogs, 25 male and 17 female, from a heterogeneous population (18 different sizes and skull types) were used in this study (Table 1). The femur and tibia bones of these dogs were used from the existing collections of Istanbul University-Cerrahpaşa Osteoarchaeology Practice and Research Centre, and the necessary ethics committee permission was obtained. (Permission of IUC Faculty of Veterinary Medicine, Unit Ethics Committee dated 01.07.2021 and numbered 2021/27).

The osteometric measurements of the femur and tibia by von den Driesch (1976), were taken as reference and a digital calliper was used to take the measurements. In the study, a total of 4 osteometric measurements of the femur and tibia were taken (figure 1). A total of 2 indices were calculated using these measurements.

Femur measurements:

Bd- Greatest distal breadth of the distal end

SD- Smallest breadth of diaphysis

Tibia measurements:

Bp- Greatest breadth of the proximal end

SD- Smallest breadth of diaphysis

Indices (Johnstone, 2004):

Femur index= Smallest breadth of diaphysis (SD)*100/ Greatest distal breadth of the distal end (Bd)

Tibia index= Smallest breadth of diaphysis (SD)*100/ Greatest breadth of the proximal end (Bp)

Figure 1. The osteometric measurements of femur and tibia (from von den Driesch 1976)

SPSS 21.0 (Version 21.0, SPSS Inc., Chicago, IL, USA). program was used for statistical calculation of osteometric measurements and calculated indices. After calculating the mean and standard deviation values of 42 adult dogs, T-test was applied to control the significance of the difference between the mean values of both sexes. After calculating the correlations between the osteometric measurements of the femur and the tibia, a

Table 1. Dog breeds and gender distribution

Breed	Number	Male	Female
French Bulldog	1	0	1
German Shepherd	6	3	3
Boxer	3	2	1
Kangal	5	4	1
Crossbreed	7	3	4
Clumber Spaniel	1	0	1
Great Dane	1	1	0
Mastiff	1	1	0
St. Bernard	2	1	1
Doberman	3	1	2
Terrier	3	2	1
Cocker Spaniel	1	1	0
Rottweiler	3	2	1
Canaan Dog	1	1	0
Irish Setter	1	0	1
Pekingese	1	1	0
Pointer	1	1	0
Siberian Husky	1	1	0

regression formulation was created to make independent estimations of both the femur "greatest distal breadth of the distal end" and the "greatest breadth of the proximal end" of the tibia. Thus, formulations thought to contribute to prosthesis applications were obtained. With these formulations, it was possible to estimate unknown distal femur or proximal tibia measurements from femur or tibia measurements. The statistical data obtained are presented in tables (Table 2 and 3) and the writing of the study was based on Nomina Anatomica Veterinaria (2017).

Results

In this study, the relationship between the distal femur and proximal tibia of dog breeds with different sizes and morphological appearances from the heterogeneous population was examined morphometrically. The femurs and tibias used were selected from individuals who did not have any bone problems. Through the statistical calculation, no homotypic variation was observed between the right and left bones of the same individual. For this reason, the mean values of the right and left bones of each individual were used. The osteometric measurements of the femur and tibia of these animals are presented in Table 2, taking into account the gender difference.

The osteometric measurements and indices of male dogs had a higher value than females. However, no statistically significant differences were observed between the mean values of males and females.

When CV values of osteometric measurements were calculated, the greatest change was observed in the measurement of "smallest breadth of diaphysis (SD)" in both genders (Table 3). CV value was calculated in the lowest index data.

High correlations were found between the osteometric measurements of each bone and with other bone measurements (Table 4). Low correlations were found between the measurement of the femur "Bd" and both

Table 2. Osteometric measurements and indices of the femur and tibia

Sex	Statistical	Bd	SD	Femur index	Bp	SD	Tibia index
	Mean	36.19	15.20	41.91	39.50	15.03	38.05
MALE	n	25	25	25	25	25	25
	SD	6.31	3.00	2.64	7.22	2.96	2.78
	Mean	34.03	14.11	41.41	38.27	13.92	36.48
FEMALE	n	17	17	17	17	17	17
	SD	4.55	2.52	3.87	5.09	1.88	2.53

Mean values in the same column are not statistically significant ($P<0.05$)

Table 3. CV values of osteometric measurements

Sex	Bd	SD	Femur index	Bp	SD	Tibia index
MALE	17.44	19.73	6.30	18.28	19.67	7.31
FEMALE	13.37	17.87	9.36	13.29	13.53	6.94

the femur and tibia indices, which were not statistically significant. Although this resulted in partially significant correlations in the measurement of "SD" of both the femur and tibia, their level were quite low. There was a very low negative correlation, not statistically significant, between the "Bp" of the tibia and the tibia index. The following equations were obtained when regression analysis was performed to estimate the distal femur and proximal tibia measurements (Femur: Bd and Tibia: Bp) participating in the formation of the articulatio genus. The level of determination (R2) of these equations was quite high.

F1=1.492+(0.867*T1) (R2=0.948)

T1=0.408+(1.093*F1) (R2=0.948)

Discussion

Dog breeds, from Yorkshire terrier to Dobermann, have different sizes and morphological structures, and show intraspecific polymorphism (Jouve et al., 2001). Therefore, although most of the common orthopaedic diseases observed in dogs are due to physical conformation and genetic predisposition (Boge et al., 2019), they are commonly found in the femur and its joints in the hind legs (Gregory et al., 1986; Knaus et al., 2003), which constitute the driving force of the trunk. These diseases are also claimed to occur in hind limbs by some previous studies (Ocal et al., 2012; Kara et al. 2018). Knee joint, which is a complex one both morphologically and functionally (De Rooster et al., 2006; Gupte et al., 2007), is a ginglymus-like compound joint (Dyce et al., 1987; Evans, 1993; Bahadır and Yıldız, 2010) due to the distal condylar structure of the femur and the plateau of the tibia (Bahadır and Yıldız, 2010). This incompatibility is corrected by intervening menisci (Evans, 1993). In the study, the morphometry of the articular surfaces of the femur and tibia, which is most emphasized in the hind limbs, was evaluated. The measurement of the smallest width of the diaphysis of the bones was also included in this assessment. Thus, the morphometry of the distal and proximal articular surfaces, which articulate in part in relation to the thinness index of the bones (Johnstone, 2004), has been associated. In these measurements, in which sexual dimorphism was not taken into account, it was observed that the CV change was the highest in the smallest diaphyseal widths of the bones, except for the measurements forming the joint. This was thought to be due to the fact that dog breeds of different sizes and morphological structures were used in the study, and therefore each breed had a different body structure.

It was observed that the difference between the measurements and indices of the femur and tibia between male and female individuals was not statistically significant. The values obtained for both genders were almost close to each other. Although sexual dimorphism refers to differences in size and form between male and female individuals (Nganvongpanit et al., 2017), it was probably due to the fact that few morphometric measurements of these bones did not reflect dimorphism. Although it was reported that breed differences, body weights and muscle mass should be taken into account in the evaluation of knee joint function using goniometric measurements, it was suggested that neither the gender of the dog nor the differences in the measured side affect the goniometric measurements of the knee joint (Sabancı and Ocal, 2016). Although information about the range of motion is widely used in many diseases and operations of the knee region (such as cruciate ligament injuries, partial patellectomy, total knee replacement) (Jandi and Schulman, 2007; Agostinho et al., 2011; Drygas et al. 2011; MacDonald et al., 2013; Skinnner et al., 2013), considering the morphometric measurements of the knee joint (Sabancı and Ocal, 2016), which is the

Table 4. Correlation analysis of indices with femur and tibia measurements

Measurements and indices	Femur			Tibia		
	Bd	SD	Femur index	Bp	SD	Tibia index
Bd	1.000	-	-	-	-	-
SD	0.924**	1.000	-	-	-	-
Femur index	0.160	0.522**	1.000	-	-	-
Bp	0.974**	0.937**	0.250	1.000	0.917**	-
SD	0.920**	0.948**	0.407**	0.917**	1.000	-
Tibia index	0.010	0.180	0.473**	-0.050	0.346*	1.000

**: P<0.01; *: P<0.05

joint most prone to orthopaedic diseases, would be beneficial. Total knee replacement operation is a well-known fact in the end-stage treatment of osteoarthritis findings, which are formed as a result of the rupture of the cranial cruciate ligament (Thitiyanaporn, 2020). We believe that knowing the morphometric measurements of the femur and tibia will increase the success in the selection of the prosthesis to be applied in these operations. It will make a great contribution especially to the evaluations at the racial level. In line with the morphometric data obtained in our study, the creation of the best regression equation for estimating the other measurement from a single bone dimension for use in possible prosthetic applications (Altunatmaz et al., 2019; Thitiyanaporn, 2020), is not only in terms of knee joint operations, but also in zoo archaeological studies. It will also contribute to the identification of individual bones. Thus, regression formulations will be available for individual identification from mixed burials.

Conclusion

As a result, while no sexual dimorphism was observed in the linear morphometric measurements and indices of the distal femur and proximal tibia, the level of determination (R2) of the regression formulas created using these measurements, is quite high. There are high correlations within and between the femur and tibia. It is believed that morphometry of the femur and tibia, which is the main element forming the knee joint, will contribute to the selection and creation of prosthesis operations such as total knee replacement.

Acknowledgements

This study benefited from the data of the first author's PhD study.

Conflict of interest

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

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Volume 11, Issue 2 July - December 2022 Page: 1-71
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