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Research Article

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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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

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

Sex	Statistical	Bd	SD	Femur index	Bp	SD	Tibia index
MALE	Mean	36.19	15.20	41.91	39.50	15.03	38.05
	n	25	25	25	25	25	25
	SD	6.31	3.00	2.64	7.22	2.96	2.78
FEMALE	Mean	34.03	14.11	41.41	38.27	13.92	36.48
	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)

measurements (Table 4). Low correlations were found between the measurement of the femur "Bd" and both 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 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

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	-	-
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

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.

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Conflict of interest

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

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Research Article

Sexual Dimorphism on the Sacrum of the Dog: A Morphometric Study

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ABSTRACT

In this study, 59 adult canine sacrums, comprised of 30 male and 29 female, with 23 different breeds were used. A total of 5 osteometric measurements were taken from each of the sacrums and a total of 3 sacrum index indices were calculated using these measurements. Statistically significant differences were observed between the mean values of osteometric measurements in males and females ($P<0.05$). However, it was determined that the difference between the mean values of the index values of males and females was not statistically significant. Hence, although linear measurements are important in terms of revealing the difference in sexual size in dimorphism, it was concluded that it would be useful to reveal different indices to make different index calculations, especially in cases where sacrum width is foregrounded.

Keywords: Dog, morphometry, sacrum, sexual dimorphism

Köpek Sacrumunda Seksüel Dimorfizm: Bir Morfometrik Çalışma

ÖZET

Bu çalışmada 23 farklı ırktan 30 erkek ve 29 dişi olmak üzere 59 yetişkin köpek sacrum'u kullanıldı. Her bir sacrum'dan toplam 5 adet osteometrik ölçüm alındı ve bu ölçümler kullanılarak toplam 3 farklı sacrum indeksi hesaplandı. Dişi ve erkeklerde osteometrik ölçümlerin ortalama değerleri arasında istatistiksel olarak anlamlı farklılıklar gözlendi ($P<0,05$). Ancak dişi ve erkeklerin indeks değerlerinin ortalama değerleri arasındaki farkın istatistiksel olarak anlamlı olmadığı belirlendi. Dolayısıyla doğrusal ölçümler dimorfizmde cinsiyete bağlı büyüklük farkını ortaya koymak açısından önemli olsa da özellikle sacrum genişliğinin ön planda olduğu durumlarda farklı indeks hesaplamaları yapmak için farklı indeksleri uygulamanın faydalı olacağı kanaatine varılmıştır.

Anahtar Kelimeler: Köpek, morfometri, sacrum, seksüel dimorfizm

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Introduction

Dogs have different sizes and visual morphologies, and skull shape is mentioned as the most important criterion in determining standard dog breeds (Onar et al., 2001) Therefore, the current skull typology has been widely used in the differentiation and identification of morphological types (Stockard, 1941; Komeyli, 1984; Brehm et al., 1985; Regedon et al., 1991; Onar, 1999; Onar et al., 2001). Another important factor affecting visual morphology are sexual differences. Sexual dimorphism refers to differences in size and form between males and females (Nganvongpanit et al., 2017). Therefore, determining the gender is considered as a first step in the creation of the biological profile (Yadav et al., 2015). Gender identification through bone morphology in dogs is quite limited (Nganvongpanit et al., 2017). Definitions have been generally made on the pelvic bones (Onodera et al., 1987; Sajjarengpong et al., 2003; Cariier, 2005; Nganvongpanit et al., 2017). It has been reported that sexual dimorphism between males and females can be revealed by using the equations created by pelvic morphometry (Nganvongpanit et al., 2017), and it would be useful to know the pelvis morphometry to identify genetic variations in postcranial morphology (Carrier, 2005). In addition to the use of pelvis morphometry in sexual dimorphism in dogs (Onodera et al., 1987; Sajjarengpong et al., 2003; Carrier, 2005; Nganvongpanit et al., 2017), there is no information about a sexual dimorphism related only to the sacrum similar to observed in humans. With the shape and morphometry, the sacrum has been the most commonly used bone, especially in humans (Ravalia and Wagh, 2015; Yadav et al., 2015; Ahankari and Ambali, 2016; Bajpai and Maiyyar, 2016;). In dogs, the assessments of this bone have been at a very limited level only in the pelvis studies (Onodera et al., 1987; Sajjarengpong et al., 2003; Carrier 2005; Nganvongpanit et al., 2017). As being a component of the pelvis, it is a fact that this bone is effective to define sexual dimorphism similar to that in humans (Ravalia and Wagh, 2015; Yadav et al. 2015; Ahankari and Ambali, 2016; Bajpai and Maiyyar, 2016).

Sacrum in dogs has occurred through the constitution of three vertebrae (Evans and Christensen, 1979; Bahadır and Yıldız, 2010). This bone, which looks like a square when viewed from the dorsum (Bahadır and Yıldız, 2010), is firmly attached through the articulatio sacroiliaca to the os ilium bones of the ossa coxae (Dyce et al., 1987). This joint plays an important role in carrying body weight and transferring propulsion from the pelvic limbs to the spine (Gregory et al., 1986; Knaus et al., 2003). The sacrum, an unpaired bone, which participates in the formation of this joint, has been considered a mechanical necessity along with the task of this joint (Çalışlar, 1995). From biomechanical point of view, the pelvic limbs musculature is responsible for kinetic energy generation, which must be transmitted to the body trunk without loss (Çalışlar, 1995). This bone participates in the formation of the pelvis (Çalışlar, 1995; Dursun, 2007; Bahadır and Yıldız, 2010; Ahankari and Ambali, 2016). Thus, it forms the dorsal boundary so-called “birth canal”, which is bounded laterally by the ossa coxae (Bahadır and Yıldız, 2010).

The sacrum, which is a dorsal component of the pelvis, is affected by all functional pressures on the pelvis (carrying weight, birth, and mode of locomotion) together with the pelvis from, and contributes to revealing the sexual size difference together with the pelvis (Csanády et al., 2019).

In this study, the relationship between sacrum morphometry and sexual dimorphism was investigated in dog breeds with different typologies and sizes. For this aim, it was evaluated whether there was a sexual dimorphism based only on the sacrum, as similar to that in humans (Ravalia and Wagh, 2015; Yadav et al., 2015 Ahankari and Ambali, 2016; Bajpai ve Maiyyar, 2016).

Material and Method

A total of 59 adult canine sacrums, including 30 males and 29 females, from a heterogeneous population (with 23 different sizes and skull types) were used in this study, (Table 1). Sacrums were derived from the existing collections of Istanbul University-Cerrahpaşa, Osteoarchaeology Practice and Research Centre, and the required ethics committee permission was obtained (Permission from İÜC, Faculty of Vet. Med. dated 24.03.2021 and numbered 60696).

Sacrum osteometric measurements were evaluated by using a digital calliper according to the criteria by von den Driesch (1976).

A total of 5 osteometric measurements of the sacrum (Fig. 1) were taken in this study. A total of 3 sacrum indices were calculated using these measurements.

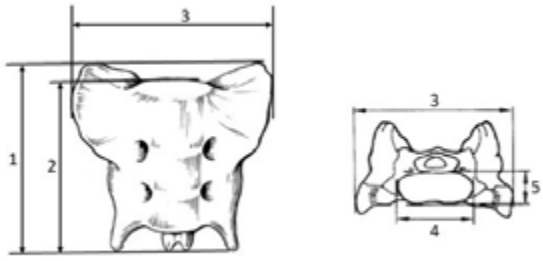

Figure 1. Sacrum osteometric measurements (from von den Driesch 1976)

Sacrum measurements: (from von den Driesch, 1976)

S1-Greatest length on the ventral side: from the cranial borders of the wings to the caudoventral border of the body of the last vertebra.

S2-Physiological length, measured between the centres of the bodies of the first and the third vertebrae.

S3-Greatest breadth (between the wings od sacrum)

S4-Greatest breadth of the facies articularis cranialis

S5-Greatest height of the facies articularis cranialis

Table 1. Dog breeds and gender distribution

Dog breeds	Male	Female
Kangal	2	2
Malaklı	2	0
St. Bernard	2	1
German Shepherd	6	4
Bernese Mountain	1	0
Rottweiler	4	1
Golden Retriever	0	2
Pointer	1	1
Shar-Pei	0	1
Doberman	2	1
Irish Setter	0	1
English Setter	0	2
Canaan Dog	1	0
Pitbull	0	1
Boxer	2	2
English Bulldog	2	0
French Bulldog	0	1
Pekingese	1	0
Chow Chow	0	2
Pomeranian	1	0
Cocker Spaniel	1	1
Dachshund	0	2
Terrier	2	4
Total	30	29

Sacrum indices:

Index-1=S3 (Greatest breadth)*100/S1 (Greatest length on the ventral side)

Index-2=S3 (Greatest breadth)*100/S2 (Physiological length)

Index-3=S5 (Greatest height of the facies articularis cranialis)*100/S1 (Greatest length on the ventral side)

The statistical analyses of the taken measurements and calculated indices were performed by SPSS 21.0 program (Version 21.0, SPSS Inc., Chicago, IL, USA). After calculating the mean and standard deviation values in males and females, the Shapiro-Wilk test was used to control for a normal distribution of the measurements. The Independent-Samples T test was applied to check the significance of the difference between the mean values of both genders that the data were normally distributed. The obtained statistical data are presented in tables, and the writing of the study is based on Nomina Anatomica Veterinaria (2017).

Results

In this study, the sacrums of the dog breeds from heterogeneous population (different sizes and skull types) were used. The sacrums were composed of 3 vertebrae, there was no fusion of the 1st caudal vertebrae. The osteometric measurements of these sacrums are presented in Table 2.

The osteometric measurements of the male dogs showed a higher value compared to female dogs. Taking into account of the usage of the heterogenous dogs with different sizes and types, these values had a fairly higher standard deviation except for the height (S5) of the Facies articularis cranialis.

Statistically significant differences were observed between the mean values of osteometric measurements in males and females ($P<0.05$). Although heterogenous dog breeds with different size and types were used, the osteometric values of the sacrum had a higher value in males. The differences of the mean values between males and females indicated the sexual size difference.

Three index calculations were performed using sacrum osteometric measurements (Table 3). Among these index calculations, the indices numbered 1 and 2 were calculated based on the width of the sacrum, and the index numbered 3 was calculated based on the height of the sacrum. The difference between the mean values of

Table 2. Sacrum osteometric measurements (mm)

Sex	Statistical	S1	S2	S3	S4	S5
MALE						
(n=30)	Mean	46.01	42.52	55.94	28.62	13.80
	SD	12.05	11.10	13.49	6.61	3.25
	Minimum	20.01	18.48	26.40	13.92	6.06
	Maximum	68.48	65.17	77.01	38.84	17.98
FEMALE						
(n=29)	Mean	40.04	36.93	48.70	25.47	12.17
	SD	8.72	7.85	9.94	4.97	2.71
	Minimum	25.83	24.05	31.50	14.40	7.40
	Maximum	54.68	52.00	66.07	34.55	17.39
	P	0.034	0.030	0.023	0.043	0.042

Table 3. Indices

Sex	Statistical	Index-1	Index-2	Index-3
MALE (n=30)	Mean	122.63	132.61	30.25
	SD	12.91	12.97	3.18
	Minimum	98.42	103.42	23.51
	Maximum	153.21	162.09	37.56
FEMALE (n=29)	Mean	123.18	133.36	30.56
	SD	17.46	19.03	3.65
	Minimum	99.47	108.45	22.73
	Maximum	181.37	202.79	41.87
	P	0.891	0.859	0.731

the index values in males and females was not statistically significant.

Discussion

As a component of the pelvis, the sacrum plays an important role in transferring the driving force from the pelvic limbs to the spine at the junction of the pelvic limb and spine (Gregory et al., 1986; Knaus et al., 2004). Therefore, it functionally effects pelvis (Kumar et al., 2018). Sacrum has been the most widely used bone in terms of sexual dimorphism, along with its shape and morphometry (Ravalia and Wagh 2015; Yadav et al., 2015; Ahankari and Ambali, 2016; Bajpai and Maiyyar, 2016; Joshi and Puranik, 2016), especially in revealing the biological profile in humans (Yadav et al., 2015). The assessments of this bone in dogs have been evaluated only at a very restricted level only in studies related to the pelvis (Onodera et al., 1987; Sajjarengpong et al., 2003; Cariier, 2005; Nganvongpanit et al., 2017). For this reason, sacrum morphometry and indices have been commonly used (Ahankari and Ambali, 2016; Bajpai and Maiyyar, 2016; Joshi and Puranik, 2016). With this aim, varying indices have been produced by these researches. With these assessments, it has been concluded that a single index cannot define the sex of sacrum with 100% accuracy, and therefore, it has been indicated that more than one index should be used to define the sex through the sacrum with 100% accuracy (Joshi and Puranik, 2016; Yadav et al., 2015). In this study, assessments were basically performed through the 3 indices. Indices numbered 1 and 2 were calculated based on the width of the sacrum, and the index number 3 was calculated based on the height of the sacrum. However, it was found that all three indices were not sufficient to reveal the sexual dimorphism.

In this study, the largest value among linear measurements was found in sacrum width as similar to those indicated by Ocal et al. (2006). This showed that in heterogeneous dog populations, even with different breeds, the width of the sacrum had a very large value compared to the length. Especially in male individuals, this linear measurement value was calculated remarkably high. The difference with females was statistically significant in terms of sexual dimorphism.

Sexual dimorphism refers to differences in size and form between male and female individuals (Nganvongpanit et al., 2017). For this reason, while it carries an important role in terms of bioarchaeology and forensic sciences in the sex identification of human skeletons (Nganvongpanit et al., 2017), it also provides great benefits in predicting the visual morphological characters of animals, and determining sex in zooarchaeological studies (Carey 1982; Grigson 1982a, 1982b; Greenfield, 2002). In the scope of the information given, it was particularly aimed to contribute to zooarchaeological studies in the present study. Although not directly, it would contribute to the importance of size differences in sex estimation based on sacrum morphometry.

Sexual size difference occurs in the sacrum because it is simultaneously affected by all functional pressures on the pelvis (carrying weight, birth, and mode of locomotion) (Csanády et al., 2019). However, calculated indices in this study were not statistically significant for the size difference. Although the effect of birth activity on the dimorphism of sacrum size has been known (Csanády et al., 2019), it was concluded that the unknown birth activities of the female dogs included in the present study were effective in evaluating the effects on the indices. The direct effect of linear measurements can be observed.

It has been known that anatomical differences are seen among dog breeds depending on the locomotion and activity form the limbs and pelvis (Schutz et al., 2009; Carlon and Hubbard, 2012). For example, in dogs with different morphological types and activities, such as Greyhounds and American Pit Bull terriers, it has been suggested that having thin or thick limbs and strong shaping of the pelvic muscles depending on locomotor anatomy cause structural anatomical differences on the pelvis, apart from sexual differences (Chase et al., 2002; Schutz et al., 2009). In this study, it is expected that 23 different dog breeds show anatomical differences in pelvis and limbs depending on their locomotion and activity. Presumably, these differences have the same effect on the sacrum, which is a component of the pelvis.

Therefore, it is thought that it may be useful to perform the evaluation of the statistical differences in linear measurements in terms of indices through grouping the different morphological types.

Conclusion

As a result, while a sexual dimorphism was observed on direct linear measurements in sacrum morphometry, sacrum indices calculated using these measurements were not statistically significant. Although linear measurements are important in terms of revealing the difference in sexual size in dimorphism, it was concluded that it would be useful to present different indices, especially to make different index calculations with sacrum width in the foreground. Nonetheless, it is believed that it would be useful to consider the sacrum indices by grouping the different morphological types of dog breeds.

Conflict of interest

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

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Research Article

Evaluation of Infrared Thermography Findings in Pseudopregnant Rabbit

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ABSTRACT

In the present study, the diagnostic efficiency of infrared thermography (IRT) implementation in pseudopregnant rabbits, the change of these images in the pseudopregnancy process, and its relationship with serum progesterone (P4) levels were examined. Fourteen healthy female rabbits of New Zealand breed were divided into two study groups. Pseudopregnancy induction was performed in the first study group with GnRH injection (0.2 ml Buserelin acetate, intramuscularly) on the 0th day of the study (Group 1, n=7). The second group (Group 2, n=7) was given placebo (0.2 ml 0.9% NaCl, intramuscularly). Rectal temperature was taken from all rabbits on days 0-5-10 and 15; eye, nasal tip, and vulvar IRT were applied; and serum P4 values were measured by Enzyme-linked immunosorbent assay (ELISA) method. In the pseudopregnant group, the temperatures of the eyes, nose, and vulva were higher on the 10th day (P<0.05). While time-dependent eye, nose, vulva, and rectal temperature changes were observed in the pseudopregnant group (P <0.05), it was not observed in the control group (P>0.05). The increase in rectal temperature was only on the 15th day in the pseudopregnant group (P<0.001). Serum P4 value was high on day 0 in Group 1 (P<0.05), but no change was observed in both groups over time (P>0.05). A significant correlation was observed between the serum P4 value and rectal temperature and the groups (r=-0.32, P<0.05; r=0.35, P<0.01). A significant difference was determined between nasal temperature and rectal temperature (r=0.28, P<0.05). As a result, IRT implementation in rabbits; It was determined that it is useful in noninvasive and rapid monitoring of body temperature and can determine the temperature increase in the eyes, nose and vulva on the 10th day of pseudopregnancy.

Keywords: Progesterone, pseudopregnancy, rabbit, thermography

Yalancı Gebe Tavşanlarda İnfrared Termografi Bulgularının Değerlendirilmesi

ÖZET

Sunulan çalışmada yalancı gebe tavşanlarda infrared termografi (IRT) uygulamalarının tanısal etkinliği, bu görüntülerin yalancı gebelik sürecindeki değişimi ve serum progesteron (P4) düzeyi ile olan ilişkisi incelendi. Yeni Zelanda ırkı, 14 adet sağlıklı dişi tavşan iki adet çalışma grubuna ayrıldı. Birinci çalışma grubuna çalışmanın 0. gününde (Grup 1, n=7) GnRH enjeksiyonu (0,2 ml Buserelin asetat, im) ile yalancı gebelik indüksiyonu yapıldı. İkinci gruba ise (Grup 2, n=7) plasebo (0,2 ml %0,9 NaCl, im) verildi. Tüm tavşanlardan 0-5-10 ve 15 günlerde rektal sıcaklık alındı;göz, burun ucu ve vulvar termografi uygulandı; Enzyme-linked immunosorbent assay (ELISA) yöntemi ile serum P4 değerleri ölçüldü. Yalancı gebe grubunda göz, burun ve vulvar bölge sıcaklığı 10. günde daha yüksekti (P<0,05). Zamana bağlı göz, burun, vulva ve rektal sıcaklık değişimi yalancı gebe grubunda görülürken (P<0,05) kontrol grubunda gözlenmedi (P>0,05). Rektal sıcaklık artışı ise yalancı gebe grubunda sadece 15. günde belirlendi (P<0,001). Grup 1'de serum P4 değeri 0. günde yüksekti (P<0,05) ancak her iki grupta zaman içinde değişim görülmedi (P>0,05). Serum P4 değeri ve rektal sıcaklık ile gruplar arasında anlamlı bir ilişki gözlendi (r=-0,32, P<0,05; r=0,35, P<0,01). Burun sıcaklığı ile rektal sıcaklık arasında anlamlı bir farklılık belirlendi (r=0,28, P<0,05). Sonuç olarak tavşanlarda IRT uygulamaları noninvaziv ve hızlı şekilde vücut sıcaklığının izleminde yararlı olup, yalancı gebeliğin 10. gününde göz, burun ve vulvadaki sıcaklık artışını belirleyebileceği belirlendi.

Anahtar kelimeler: Progesteron, tavşan, termografi, yalancı gebelik

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Introduction

Rabbits have been bred for meat production and experimental animals in scientific research for a long time, but they have been frequently seen as pet animals in veterinary clinics. Although their reproductive physiology depends on day length, environmental temperature, nutrition, and social factors (Bell, 1999), the rabbits bred in the laboratory environment reflect the symptoms more stable. By the neurohumoral pathway due to mating, jumping movements, or carrying stress, ovulation stimulates (Dal Bosco et al., 2011; Donnelly, 2013; Carter et al., 2016). Within 1-2 hours after stimulation, serum LH level reaches its peak level and reaches 8-10 ovulation takes place within hours. However, spontaneous ovulation can also be seen under good care and feeding conditions (Bekyürek, 2010). In each ovulation, an average of 6-10 oocytes reaches the oviduct (McNitt et al., 1996). In the condition of non-fertilization, pseudopregnancy lasting 15-18 days may be encountered (McNitt et al., 1996; Donnelly, 2013). There is no difference in serum progesterone levels until luteal regression begins in pregnant and pseudopregnant rabbits (Ptaszynska, 2001). Corpora lutea that occurs in pseudopregnancy persists for 10-12 days and causes external deposits such as pregnancy behaviors and nest preparation for birth with the effect of estrogen, P4 and prolactin hormones. Observed behavioral changes also disappear with luteal regression (Donnelly, 2013).

Infrared thermography (IRT) is a real-time, non-invasive, and side-effect-free auxiliary diagnostic tool (Huynh, 2019). In addition, the compatibility of eye temperature with rectal and vaginal measurements was also investigated (Sykes et al., 2006; Willard et al., 2006). Its non-invasive usage has made it more accessible to detect ovulation and pregnancy in Asian elephants, rhinos, and giraffes (Durrant et al., 2006; Hilsberg-Merz, 2008). Moreover, the distinction between pregnant and pseudopregnant pandas could be performed (Durrant et al., 2006). It is also effective in detecting estrus and determining the appropriate insemination time with IRT in the farm animals, dogs, and chinchillas (Osawa et al., 2004; Sykes et al., 2006; Talukder et al., 2015; Olğaç et al., 2017; De Freitas et al., 2018; Façanha et al., 2018).

Although many IRT results belong to different species in literature, limited data are seen about the rabbits. Therefore, this study was aimed to evaluate the diagnostic efficiency of IRT taken from different body parts of rabbits undergoing pseudopregnancy.

Materials and Methods

In the study, 14 New Zealand breed female rabbits, 1-3 years old, housed in an individual cage setup and known to be healthy in general, and gynecological examinations were used. All animals were housed in individual wire mesh cages under controlled temperature (18-24°C) and light conditions during the study. They were fed ad-libitum water and pellet calf rearer feed. Animals were randomly divided into two study groups (Group 1 and 2). During the study, general care and all clinical practices were carried out according to the regulations of Aydın Adnan Menderes University Experimental Animals Application and Research Center and in line with the relevant ethics committee decision (64583101/2019/007).

In the study, rabbits with pseudopregnancy induction were included in Group 1 (n=7), while rabbits without any induction were considered Group 2 (n=7). Accordingly, on the 0^{th} day of the study, 0.8 mcg buserelin acetate (Buserin, Alke®, Turkey) was injected intramuscularly to stimulate pseudopregnancy in Group 1. In Group 2, intramuscularly placebo injection (0.2 ml of 0.9% NaCl) was used on the same day. All IRT imaging from different body points, measuring rectal temperature with degrees, and collecting venous blood samples are included in the 0-5-10-15 days of the study. The procedures were repeated 70 times for all animals on the respective days, 280 times.

Taking IRT Samples

A mobile thermal camera (Flir E6, Flir Systems AB®-Sweden) system recorded thermal images. Attention was paid to keeping the ambient conditions (temperature change, airflow, humidity, etc.) for the images' standardization. During the measurements, uniformity was ensured in the images under the environment's temperature, in which the device was single and center oriented. During the IRT, care was taken to ensure an average of 1 meter between the camera and the rabbit. During thermal imaging, the rabbits were taken from their cages not to cause stress, and they were rested on the table on which the shooting would take place for about 10 minutes. At the end of the waiting period, thermal images of the eye, nose tip, and vulvar region were taken from the rabbits in both study groups. In eyeshots, images were taken from the lateral line, regardless of the direction, while the animals were not stressed. In nasal tip extractions, the nasal tip was fully visualized (upright/slightly oblique), taking into account the stress conditions. In the vulvar region shots, the rabbits were taken in restraint by lying on their backs not to cause stress. Finally, the tail tip was directed ventrally, the vulva and perineum region were visualized, and the shooting was taken. All measurements were completed in approximately 15 minutes for each rabbit.

Serum Progesterone Measurements

Venous blood samples were taken to measure serum progesterone value from the ear vein (Vena auricularis) after the images were taken on the days (0-5-10-15. days) when images were taken with a thermal infrared camera. The serum extracted from the samples were stored at -20 degrees until the progesterone measurement was made. At the end of the study, serum P4 levels were determined from the serum samples by the Enzyme Linked Immunosorbent-Assay method (ELISA) using the commercial Rabbit (PROG) ELISA test kit (Shanghai Sunred Biological Technology) was used.

Body temperature values measured on IRT images were compared between Group 1 and Group 2, and the curves of change over time for each group were extracted. The

obtained data were evaluated using the SPSS 22.0 (SPSS Inc. Chicago, IL, USA) package program. Shapiro-Wilk test was used to analyze whether the data met normal distribution assumptions. Pairwise comparisons were evaluated with the Independent Groups t-test. Repeated Measures ANOVA was used to detect time-dependent changes within the group. Pearson Correlation Analysis was used to determine the relationship between variables. The tables, graphics, and results are given as mean±standard error of mean (SEM). Statistical significance was accepted as $P < 0.05$.

Results

After the intramuscular injection of 0.2 ml of GnRH, no complications were encountered to induce pseudopregnancy in rabbits. Imaging was performed smoothly and quickly thanks to the elimination of stress factors. It was observed that around 12-15 days, pseudopregnant rabbits pulled their underbelly wool to a corner of the cage as if they were going to give birth, and exhibited the behavior of preparing for birth.

At the end of the study, the average temperature values are taken from the eye, nose, and vulva region with the help of a thermal camera are presented in Table 1, Table 2, Table 3, Figure 1, Figure 2, Figure 3. Accordingly, on the 10th day of pseuopregnancy, a significant temperature increase was observed in the IRT images taken from the eye, nose, and vulva region ($P < 0.05$), especially from the eye ($P < 0.01$). At the end of the study, the rectal temperatures by digital thermometer are presented in Table 4 and Figure 4. Accordingly, a significant difference was detected between the body temperatures taken on the 15th day ($P < 0.05$). The serum P4 levels of the rabbits in the pseudopregnant group were 135.57±19.05 pmol/L on day 0, 122.43±14.95 pmol/L on Day 5, 125.29±9.61 pmol/L on Day 10, and was measured as 120.00±10.11 pmol/L on the 15th day (Table 5, Figure 5). There was no statistical difference in serum P4 concentrations between

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

Day	n	Pseudopregnant	Control	P
		$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$	
0	7	34.34±0.29^{ab}	33.95±0.30	0.382
5	7	34.22±0.32^{ab}	34.51±0.26	0.509
10	7	35.12±0.14^{a}	34.18±0.23	**0.006**
15	7	33.87±0.22^{b}	34.05±0.12	0.487
P_{ANOVA}		**0.016**	0.407	

$^{a.b}$: Different letters at the same column show statistical differences
P<0.05: Significant difference between study groups

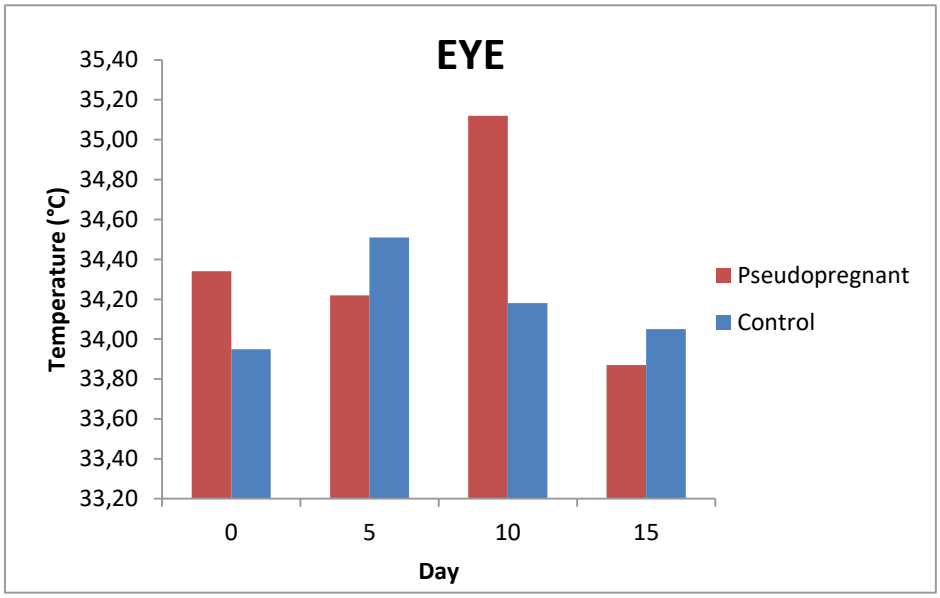

Figure 1. The mean eye temperatures of study groups (°C).

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

Days	n	Pseudopregnant	Control	P
		$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$	
0	7	30.77±0.68^{ab}	30.84±0.61	0.940
5	7	28.84±0.96^{b}	28.88±0.96	0.975
10	7	31.80±0.37^{a}	30.20±0.59	**0.041**
15	7	30.35±0.67^{ab}	30.12±0.63	0.809
P_{ANOVA}		**0.050**	0.299	

$^{a.b}$: Different letters at the same column show statistical differences
P<0.05: Significant difference between study groups

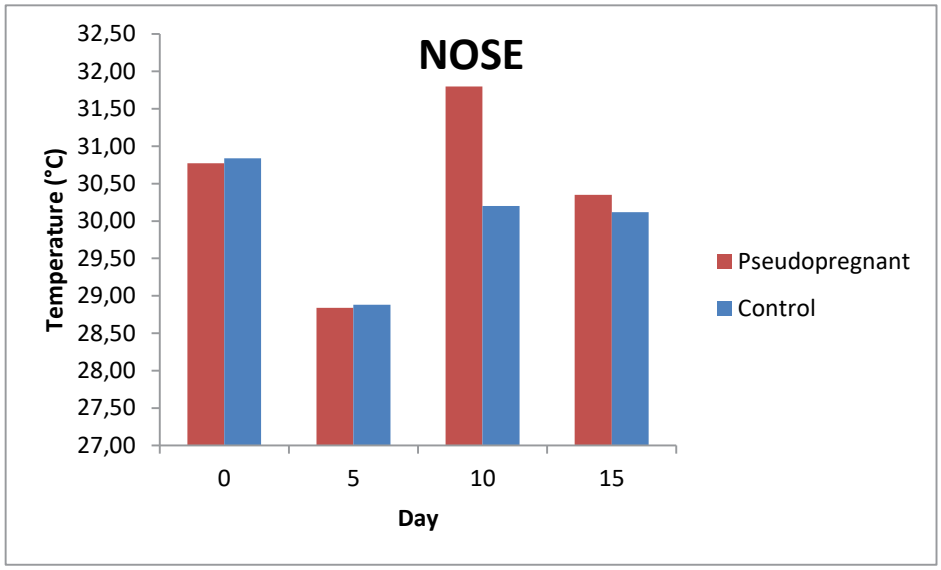

Figure 2. The mean nasal temperatures of study groups (°C).

Table 3. The mean vulvar temperatures of study groups (°C).

Day	n	Pseudopregnant	Control	P
		$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$	
0	7	30.82±1.16^{b}	32.38±0.54	0.249
5	7	32.61±0.68^{ab}	32.11±1.06	0.699
10	7	34.58±0.38^{a}	31.41±1.01	**0.012**
15	7	32.17±1.02^{ab}	32.61±0.52	0.707
P_{ANOVA}		**0.041**	0.756	

$^{a.b}$: Different letters at the same column show statistical differences
P<0.05: Significant difference between study groups

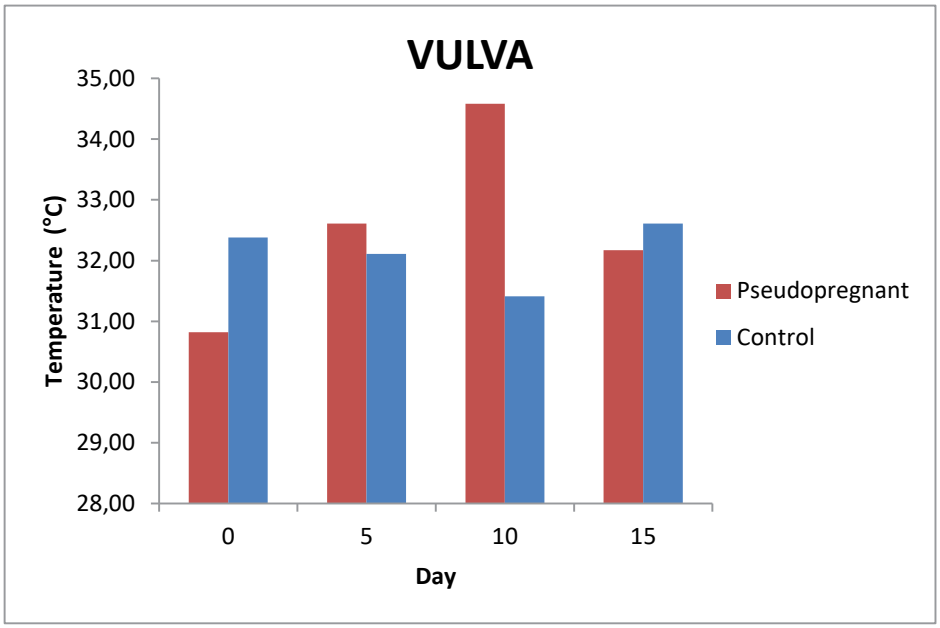

Figure 3. The mean vulvar temperatures of study groups (°C).

Table 4. The mean rectal temperatures of study groups (°C).

Day	n	Pseudopregnant	Control	P
		$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$	
0	7	38.35±0.30^{b}	38.14±0.11	0.530
5	7	37.88±0.24^{b}	38.22±0.19	0.287
10	7	38.80±0.30^{ab}	38.20±0.20	0.125
15	7	39.50±0.15^{a}	37.62±0.37	**0.001**
P_{ANOVA}		**0.002**	0.276	

$^{a.b}$: Different letters at the same column show statistical differences
P<0.05: Significant difference between study groups

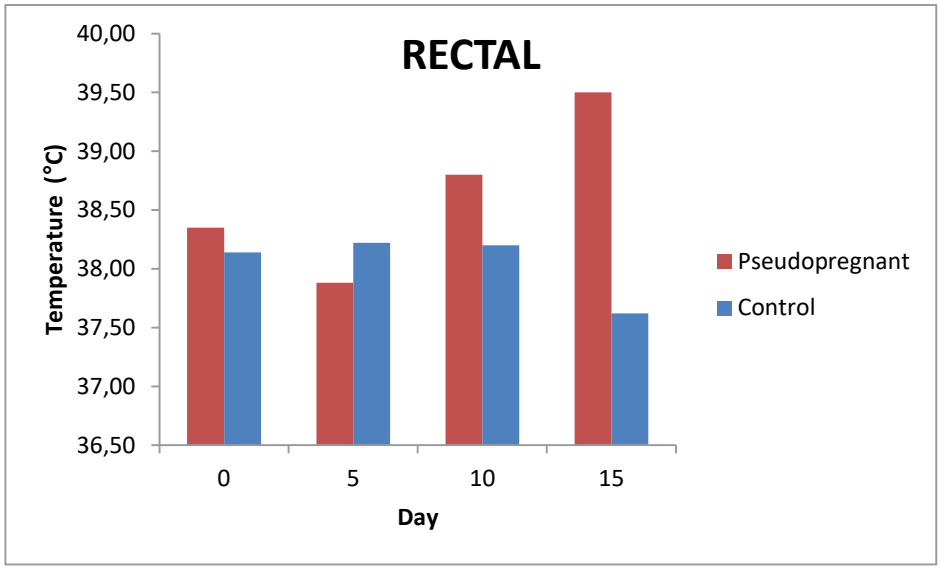

Figure 4. The mean rectal temperatures of study groups (°C).

Table 5. The mean progesterone values of study groups (pmol/L).

Day	n	Pseudopregnant $\bar{X} \pm S_{\bar{x}}$	Control $\bar{X} \pm S_{\bar{x}}$	P
0	7	135.57±19.05	83.57±6.02	**0.023**
5	7	122.43±14.95	105.71±3.84	0.300
10	7	125.29±9.61	109.71±15.77	0.416
15	7	120.00±10.11	116.29±12.94	0.825
P_{ANOVA}		0.867	0.189	

[a.b]: Different letters at the same column show statistical differences
P<0.05: Significant difference between study groups

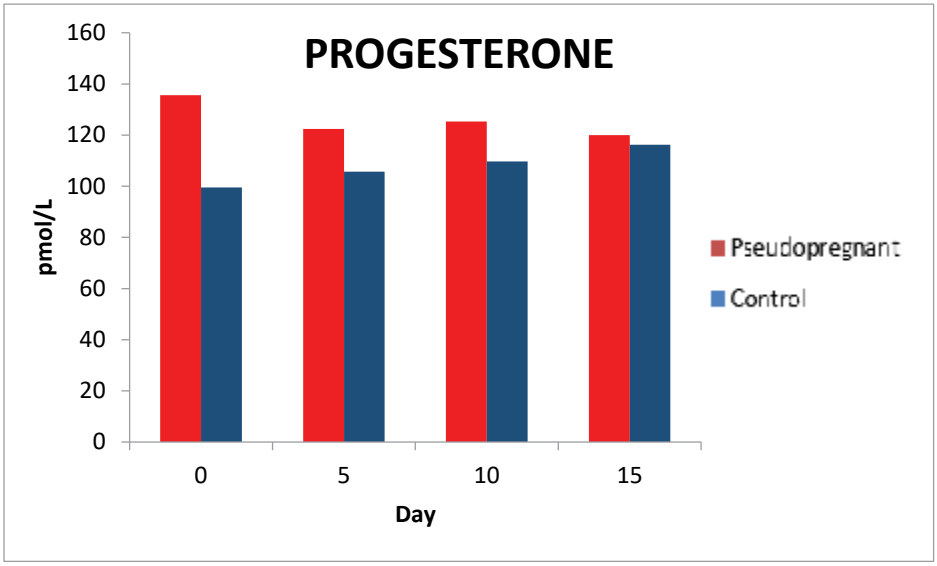

Figure 5. The mean progesterone values of pseudopregnant and control groups (pmol/L)

days (P>0.05). The serum P4 levels of the rabbits in the control group were 83.57±6.02 on day 0, 105.71±3.84 on Day 5, 109.71±15.77 on Day 10, and 116.29±12.94 on Day 15. In addition, there was no statistical difference in serum P4 levels between days in the control group (P >0.05). On the 0th day of the study, serum progesterone value was significantly higher in the pseudopregnant group (P<0.05).

There is a low (+) positive correlation between progesterone level and day (0.09). A low negative relationship (-0.08, -0.16, -0.03, -0.04, respectively) was observed between progesterone and eye, nose, vulva, and rectal temperature. There was a significant (P<0.05) moderate negative (-0.32) relationship between progesterone and the group. There was a significant (P<0.01) positive moderate (0.35) relationship between the rectal temperature and the group. There was a low positive correlation (0.18, 0.04, 0.08, respectively) between rectal temperature and day, eye, and vulva temperature. A positive and moderate (0.28) relationship was found between the rectal and nasal temperature (P<0.05). There was a low positive correlation (0.09, 0.14, 0.23, 0.13, respectively) between the measured from the vulva with a thermal camera and the group, day, eye, and nose temperatures. There was a low positive correlation between nose temperature and group, day and eye temperatures (0.10, 0.02, 0.10), respectively. Considering the eye temperature, the group is positively low (0.14); there was a low negative correlation with the day (-0.04) (Table 6).

Discussion

The main purpose of this study is to use non-invasive and easy-to-apply IRT images to diagnose pseudopregnancy in rabbits. Although the rate of pseudopregnancy is expected to be relatively low in female rabbits kept in individual cages and raised away from the presence of males, spontaneous ovulation and pseudopregnancy can be seen for different reasons (Bekyürek, 2010; Donnelly, 2013; Carter et al., 2016). Despite the use of drugs compatible with the literature for induction (0.2 ml Buserelin, intramuscularly); it is noteworthy

Table 6. Correlations between temperatures and serum progesterone values in all rabbits

	Group	Day	Eye	Nose	Vulva	Rectal	Progesterone
Group	-	-	-	-	-	-	-
Day	0.00	-	-	-	-	-	-
Eye	0.14	-0.04		-	-	-	-
Nose	0.10	0.02	0.10		-	-	-
Vulva	0.09	0.14	0.23	0.13	-	-	-
Rectal	**0.35****	0.18	0.04	**0.28***	0.08	-	-
Progesterone	**-0.32***	0.09	-0.08	-0.16	-0.03	-0.04	-

*P<0.05; **P<0.01: Showing significant correlations

that the difference between the P4 values of the two study groups was statistically seen only on the 0th day, and the presence of a numerical increase in the pseudopregnant group on the other days, which did not make a difference. Although fur plucking and nest-building behaviors were observed during the study, this suggests the existence of possible individuals with spontaneous ovulation on different days. It has been reported that 23% of pseudopregnancy are seen even in rabbit populations in individual cages (Carter et al., 2016). Pseudopregnancy could be observed in 23% of rabbits housed in groups and in hierarchical order (Rommers et al., 2006). Rabbits that remain passive by leaving the group and switching to a single cage system may also have pseudopregnancy (Carter et al., 2016). Researchers have provided the induction of ovulation by mechanical stimulation of the vagina, mating with a vasectomized male or injections of GnRH, LH, and hCG to investigate ovulation and corpus luteum (CL) physiology (Zavos et al., 1998). The hormone measurements made to determine the success of the induction process were made with ELISA at the end of the study, instead of using the rapid P4 kit during the study, caused this situation to be noticed later. Again, this situation can be investigated with more animals in the future. In future studies, it will be helpful to confirm that all material is not in the luteal phase using perineal inspection, vaginal cytology, and rapid hormone measurements. However, the possibility that the physician can stimulate ovulation during manipulations during this clinical evaluation should also be considered. Pregnancy behaviors can be observed in pseudopregnant rabbits under the influence of estrogen, progesterone, and prolactin hormones (Donnelly, 2013). Among all these physiological changes, it is expected that the diagnostic efficiency will be limited since the temperature changes due to pseudopregnancy will occur in a narrower temperature range. The diagnosis of pseudopregnancy in the use of IRT in the reproductive examinations of domestic and wild animals is a new application, and a similar application has been investigated using abdominal scans in pandas and dogs (Durrant et al. 2004; 2006). At the end of this research, it was seen that especially the shots in pregnant pandas were more sensitive than dogs.

In thermographic studies in rabbits, it is known that the most suitable attraction areas are the eyes and ears (Redaelli et al., 2014). However, the study aimed to investigate alternative regions, and the nasal tip and vulvar region were added to this. Additionally, hiding abdomen postures in the cage also arose the need for these measurements to be focused on the face, and the measurements were carried out considering these conditions in the present study. Measurements of vulvar temperature changes can be challenging, mainly since they cover the perineum with their tails. Unlike facial imaging, it can be thought that there are manipulations that can trigger stress and affect the results, taking the animals out, tilting them, and pulling the tail to make the region visible. In order to prevent such negativities, it will be effective to monitor the animals by the same person and handle them duly without causing stress.

When this study is evaluated within the data obtained with the number of animals available, the most sensitive period for the eye, nose and vulvar region thermal shots in terms of diagnostics is the 10th day. In pseudopregnancy, the CL is functional for 16-18 days, and thus the CL of pseudopregnant rabbits begins to regress due to increased PGF-2α luteal response (Dugré et al., 1989). Considering that the CL formed in pseudopregnancy persists for 10-12 days (Donnelly, 2013) and that increased P4 activates the catabolic metabolism and raises the basal body temperature (Regidor, 2014), this situation is compatible with the literature. It was determined that the significant temperature increase observed on the 10th day in the pseudopregnant group was mainly in the eyes, followed by the vulva and nasal region. A mature CL will respond to luteolytic agents in the relevant period. The high correlation between eye temperature and the rectal temperature has been detected in different species, and it is known to facilitate remote thermal evaluations (Redaelli et al., 2014), additionally, to measure stress response (Ludwig et al.,

2007).

An increase of more than 2.33 ^{0}C was found in thermal images made due to hyperemia in the vulva in Chinchillas during the estrus period (Polit et al., 2018). Similarly, IRT can determine the vulvar temperature increase during estrus in rabbits and other livestock (Osawa et al., 2004; De Freitas et al., 2018; Façanha et al., 2018). However, it was also stated that perivulvar temperature measurements were not successful in pregnancy diagnosis in cows (Radigonda et al., 2017). Vulvar hyperemia is seen in estrus in rabbits (Bekyürek, 2010); however, it is not among the pseudopregnancy findings. Despite this, in the presented thesis study, a temperature increase of 3.17 ^{0}C was detected on the 10th day of pseudopregnancy compared to the control group. Although there is no hyperemia or cyanosis in the vulva, this temperature increase is expected to be in parallel with the measurements in the eyes and nose, but the limited literature on the subject creates difficulties in interpretation. Due to the excessive hairiness of the perineal region in vulvar measurements, the application is not practical, and this situation is similar to previous studies (Polit et al., 2018). When measuring in future studies, it should be kept in mind that the hairs in the area, the fecal residue found on the rectum's outer surface and cannot be cleaned entirely, can create artifacts and prevent temperature measurement.

When the rectal temperature measurements taken in the study were examined, it was seen that they did not show similar curves with the IRT results. A significant temperature increase in the pseudopregnant group was observed on the 15th day. As shown in the graph in Figure 4, the temperature increase on the 10th and 15th days in the pseudopregnant group was evident only on the 15th day ($P<0.001$). It is noteworthy that temperature changes were detected in two different methods on different days. Although rectal temperature measurement is an effortless application, the reason for this incompatibility may be that the temperature is in contact with feces in the rectum and not enough in contact with the mucosa. In addition, different from far-IRT, it was thought that the stress caused by manipulations in rectal degree measurement might have affected the results. Another problem is the risk of ovulation stimulation during rectal temperature measurements. Another possible reason for the control group's P4 values to be close to the pseudopregnant group during the study is the uncontrolled stimulation during these measurements.

By decreasing of the P4 level after the Days 10-12, as seen in Table 6, the eye, nose, and rectal temperature continue to increase with the decrease in days showing a negative correlation between progesterone and eye, nose, and rectal temperature. The negative correlation between the P4 level and the groups is a finding parallel to the fact that the P4 level in the pseudopregnant group increased until the 10th day and then decreased. Regarding P4 measurements made in similar studies, it has been reported in the literature that serum progesterone levels in rabbits are high during pseudopregnancy, and the upper limit values measured are around 30 ng/ml. However, with the regression of the corpus luteum, there is a rapid decrease in progesterone, and it has been stated that it falls below 0.6 ng/ml (Orstead et al., 1988; Maranesi et al., 2018). Therefore, generally, it is seen that the P4 measurement unit is ng/ml in literature studies (Maranesi et al., 2018). However, due to the kits used in the presented study, calculations were made at the pmol/L level. Since this unit is mentioned in the previous literature (Fragalà et al., 2015), the current measurement unit is ng/ml (Orstead et al., 1988; Maranesi et al., 2018) can be challenging to discuss the findings.

Conclusion

As a result, temperature rise can be easily determined by IRT taken from different body parts on the 10th day of pseudopregnancy rabbits. This condition should be considered a preliminary diagnosis finding to distinguish it from other individuals and should be evaluated with other clinical / laboratory measurements. Depending on the species, the findings should be carefully examined against the possibility of pseudopregnancy even in separate breedings with ovulation stimulation during handlings. Although IRT measurements are a helpful method like a rabbit, they are open to new regulations and improvements. Therefore, investigating optimum conditions and making preliminary preparations will benefit more precise and practical measurements.

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Conflict of interest

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

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Research Article

The Use of Three-Dimensional Models for the Teaching Anatomical Structures in Biology Courses

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ABSTRACT

The study aimed to evaluate the effects on the success of students in examination by using a three-dimensional plastic model and a three-dimensional digital computer application for the teaching of the subject of "Eye anatomy" under the title of "Sensory organs". The study was conducted on the three groups of high school students (N=43). The groups were split into different laboratories for freelance work. The first group was given standard lecture notes, the second group was given 3D plastic eye models and the third group was given a 3D digital eye model application in their computer environment and they were left to work for equal periods. Pre-test and post-test achievement exams were used as data collection tools to measure the achievement levels of the students on the subject of "Eye anatomy". At the end of this study, no significant difference was found among the groups according to the statistical analysis results. It is assumed that the results may be related to the study habits of the students for the university entrance exam in Turkey; the characteristics of the three-dimensional models used, or the fact that only short-term memory has been tested. So, extensive research is needed to consider these issues.

Keywords: Anatomy, education, three-dimensional model.

Biyoloji Derslerinde Anatomik Yapıların Öğretimi İçin Üç Boyutlu Modellerin Kullanılması

ÖZET

Çalışmada «Duyu organları» başlığı altında «Göz anatomisi» konusunun öğretimi için üç boyutlu plastik model ve üç boyutlu dijital bilgisayar uygulaması kullanılarak öğrencilerin sınav başarısına etkilerinin incelenmesi amaçlanmıştır. Araştırma lise öğrencilerinden oluşan üç grup (N=43) ile yürütülmüştür. Gruplar, serbest çalışma için farklı laboratuvarlara ayrıldı. Birinci gruba standart ders notları, ikinci gruba 3 boyutlu plastik göz modelleri ve üçüncü gruba bilgisayar ortamında 3 boyutlu dijital göz modeli uygulaması verilerek eşit sürelerde çalışmaya bırakılmıştır. Öğrencilerin "Göz anatomisi" konusundaki başarı düzeylerini ölçmek için veri toplama aracı olarak ön test ve son test başarı sınavları kullanılmıştır. Bu çalışma sonunda öğrencilerin sınav başarılarının değerlendirildiği istatistiksel analiz sonuçlarına göre gruplar arasında anlamlı bir farklılık bulunmamıştır. Sonuçların, öğrencilerin Türkiye'deki üniversite giriş sınavına yönelik çalışma alışkanlıkları, kullanılan üç boyutlu modellerin özellikleri veya sadece kısa süreli belleğin test edilmiş olması ile ilgili olabileceği düşünülmektedir. Bu konuları göz önünde bulundurarak gelişmiş araştırmalara da ihtiyaç vardır.

Anahtar sözcükler: Anatomi, eğitim, üç boyutlu model

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Introduction

Learning is expressed as the reception and processing of stimuli through sense organs. Learning style is the characteristic that shows the individual's preferences for learning. Individual variances of learning style are thought to be shaped by the individual's innate differences in perception and thought (Ünal and Kavalcı, 2016). In recent years, the number of studies on the variability in the learning styles of different generations has been quite high. Generation "Z" individuals born in the 2000s and later years are in the middle of rapidly developing technology and live together with it (Akdemir et al., 2013; Toruntay, 2011). This generation carries technology to every stage of life. It has been determined that the generation "Z" individuals use perceptual-intuitive learning styles intensively. Perceptual learners mostly prefer to use their senses, factual information, and sequential information related to real life. Symbols and concepts are interesting, while details are disliked (Ardıç and Altun, 2017). It is seen in studies that linear thinking and learning processes, which are currently dominant in the education system, are not preferred by students (Prensky, 2001; Şahin, 2009). Most students study the subjects only by memorization method in biological education, so they have difficulty making connections between subjects. The results of this situation draw attention when the results of the University Exam in our country are surveyed. It is seen that the average net point of biology is 1.3 in 13 questions (ÖSMY 2022).

Considering these data, the need emerges to associate learning and teaching strategies in the biology curriculum with learning methods unique to generations. It is stated that students have difficulty in understanding, especially because anatomical structures such as the sensory system contain many abstract and complex concepts, and three-dimensional models can facilitate the understanding of such subjects (Düşkün and Ünal, 2016). Anatomy education is the most essential subject in the field of health sciences, and this starts in the pre-university education. Today, studies are showing the coexistence and interaction of biology education at the high school, and university levels and the need for its reorganisation are frequently encountered (Andariana et al., 2020; Caro et al., 2018; Labov et al., 2010; Jensen et al., 2013; Shegog et al., 2012; Taraban et al., 2015). Studies conducted in recent years also show that 3D printers

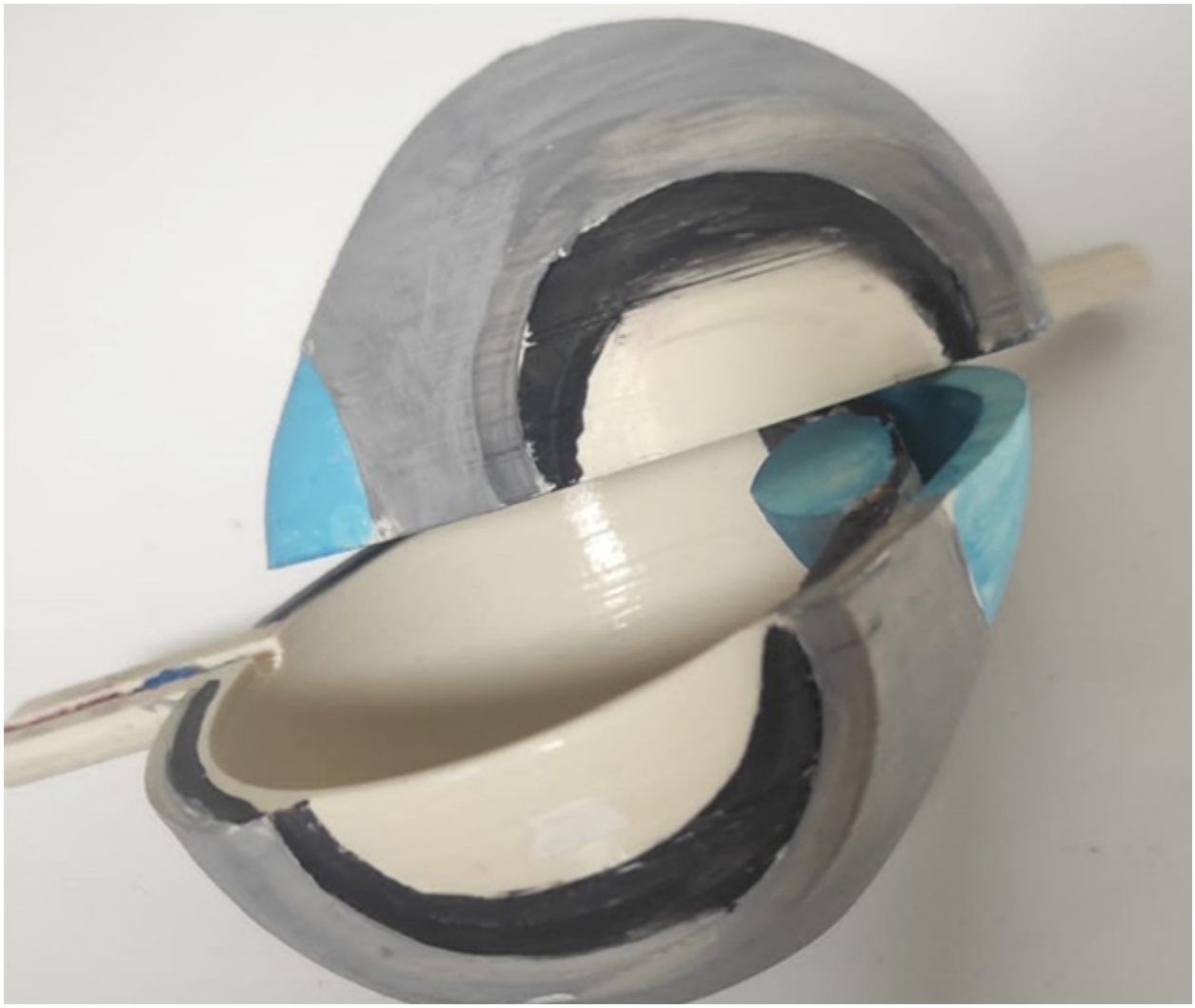

Figure 1. Printed three-dimensional eye model

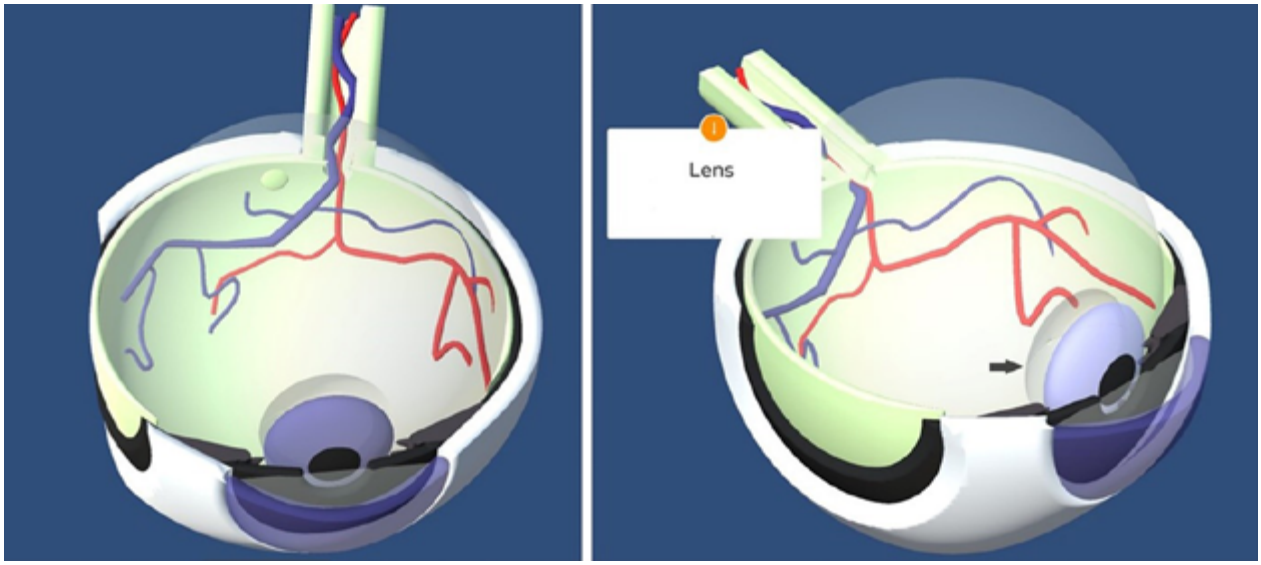

Figure 2. The 3D digital eye model application

are helpful in subjects that students have difficulty in understanding (Bakıcı et al., 2021; Bejdic et al., 2021; Düşkün and Ünal, 2016; Fancovicova and Procop, 2014; Yılgör Huri and Oto, 2022). It is easier to explain complex concepts to students, attract students' attention and participate in the lesson more effectively, and in-class interaction can be easier using 3D technologies, which have many application examples such as the printing of the molecular models in biology (Kökhan and Özcan, 2018). Elangovan et al. (2014) studied with 136 high school students to explain the subject of "cell division" with the classical method and 3D model support in the biology lesson. As a result of the research, they observed that the lesson's success and the percentage of recall were quite advanced in the group using 3D printed models. In addition, the importance of education methods outside the laboratory environment has been more acknowledging, especially during the current COVID-19 pandemic times (Davis and Pinedo, 2021).

This study aimed to observe the effect of using different methods in teaching anatomical subjects in biology lessons on academic achievement. Especially in recent years, the fact that three-dimensional (3D) models have gained an essential place in the education system and students use technology intensively in their daily lives constitutes the basis of this study.

Materials and Methods

A total of 43 high school students divided into three groups, participated in this study. The first group was given standard lecture notes, the second group was assigned 3D plastic eye models and the third group was given a 3D digital eye model application on their computers during their freelance work time. All students were from the same grade, and attention was paid to ensure that the students' academic achievements forming the groups were close to each other.

The digital and solid 3D models were prepared before starting the study. Three-dimensional plastic eye models were designed with a 3D printer. For this purpose, a free-to-use three-dimensional eye model file was saved from the address "https://grabcad.com/". The file was saved in the solid model file format by making some rearrangements in Solidworks and other CAD programs to obtain the same anatomical eye structure and fulfil the requirements of the three-dimensional printer. These files were transferred to a three-dimensional printer (Rigid3D (Sutaş, İzmir, Turkey). A total of 15 three-dimensional eye models were printed. In these models, the parts and layers of the eye were painted with acrylic paint close to the original colours and were made ready for the study (Figure 1). The 3D digital eye model application was prepared by SK to use in the

Table 1. Statistical analysis results of the groups' pre-application (pre-test) and post-application (post-test) exam results

	Classical method (n=15)	3D printing model (n=14)	3D digital model (n=14)	P
The pre-test scores	33.67±14.45	29.14±14.66	35.57±12.40	**0.459**
The post-test scores	59.47±14.92	56.64±15.30	59.29±13.34	**0.960**
P	**0.000**	**0.000**	**0.000**	

* Values are presented as mean value ± standard deviation.
P<0.05: Significant difference between study groups

computer environment. In this application, the 3D model file mentioned above was used. This application was coded using the "Unity 2019.2.1f1" program to work on "Android OS" and "Windows PC" platforms. When the application was launched on any of these platforms, the eyeball model opened in the centre of the screen. The user was able to perform interactively by clicking or touching the model. When the relevant part of the three-dimensional model was touched, the colour of the region changed to (R155 G155 B255), and a popup window opened containing the name of the part and brief information about the part, and zooming-out and rotation were done on the 3D model with "pinch and drag" movements (Figure 2).

Within the scope of the research, it was tried to determine the cause-effect relationships of the variables in different learning environments by making their applications within other groups. In this context, the achievement of the students was assessed with the prepared pre-test and post-test questionnaire. The tests consist of 20 questions, which were parallel to the curriculum and were prepared by taking into account the questions that were already asked in the university exam.

After the study groups were formed, all the students were gathered in the same hall and were informed about the subject. A pre-test was conducted on all the students before undergoing this experimental research process. After this application, while all the students were together, the subject was taught with classical lectures and the question-answer method. The groups were then split into different laboratories for freelance work. One group was given lecture notes, the second group was given plastic eye models, and the third group was given a 3D digital eye model application in the computer system. They were left to work for free for equal periods. After the time was finished, a post-test was applied to all the students at the same time, and the results were subjected to statistical analysis.

Statistical analysis of the results was made using SPSS for Windows program. The normal distribution of the groups' pre-test and post-test success scores was checked with the Shapiro-Wilks test. Since all data showed normal distribution, the subsequent analyses were planned as parametric analyses. First of all, whether there was a significant difference between the groups' academic achievement pre-test and post-test scores was checked with the t-test for dependent variables. A one-way analysis of variance (ANOVA) was used to check whether there was a significant difference between the academic achievement pre-test scores of the groups or not.

Results

The statistical analysis results of the three groups' pre-test and post-test exam results (classical method, 3D eye models and digital 3D computer application) are presented in Table 1.

There are statistically significant increases between the pre-test and post-test scores in all three groups. The pre-test exam scores of the control group students working with the textbook were 33.67, while this value increased to 59.47 after the application. While the average pre-test result of the experimental group of students who made the model work was 29.14, this value became 56.64 after the application. In the experimental group formed by students working with 3D models in the computer environment, these values were 35.57 and 59.29, respectively.

There was no statistical difference between the exam scores of the three groups both before the application (P: 0.459) and after the application (P: 0.960).

Discussion

The study groups must have similar characteristics to compare the educational methods. Attention was paid to ensuring that the academic achievement levels were similar while creating the groups. Thus, no statistical differences were observed between the pre-test exam results of the groups. Therefore, we may accept that the groups were formed as a homogeneous level of attainment. All the students were also informed about the experiment at the same time, and a common pre-course of all groups and a free-study course were applied simultaneously. Thus, according to the study results, the methods used in the formation of all the three groups also had positive effects on learning.

It is generally stated that the use of 3D models is more effective on student achievement than other methods (Elangovan et al., 2014, Park et al., 2019; Şahin, 2009). For this reason, it can be thought that the generation that widely uses digital technology may provide more effective learning through 3D modelling methods. But, there was no statistical difference between the exam scores of the three groups after using the different methods of teaching to learn eye anatomy. The low number of students in the groups may be a factor in why the results were not in the desired direction in this study. On the other hand, the design of the 3D models used in this study may also have affected the study results. The ease of use of the designs cannot be evaluated, either, since no feedback was received on this issue, or the possibility of comparison with different models cannot be found in the study. The possible third factor to take care of is students' study habits with classical methods, especially for exams in our country, which can also be the most critical factor. Park et al. (2019) stated that 82.5% of the students who used a 3D atlas in their studies found 3D atlas easier than a 2D atlas, and 90% of them stated that a 3D atlas is a more understandable-instructional method. However, only 42.5% of these students stated that a 3D atlas could completely replace 2D in the next 10 years (Park et al., 2019), suggesting that there is a more hesitant approach in the transition from classical methods to 3D methods. Fančovičova and Prokop (2014) also offer that alternative methods should not conflict with the traditional methods in anatomy education. Because the result of their study showed that the combination of cadaver dissection with modern innovative methods was more effective for obtaining anatomy knowledge than the use of only one method.

These results may also support the last comments about the results of this study. It may be expected that students will get successful results in test-type exams with the classical course methods that they are accustomed to. For this reason, increasing the number of samples in further studies and repeating the study with more advanced 3D models will contribute to such studies.

It may be accepted that the used pre and post-test exams in this study evaluate short-term learning. Performing permanence tests to evaluate the data that the educational environment created with classical methods and 3D models providing long-term learning may also be effective in detailing such studies. Because it has been reported that it is easier to obtain deep anatomical information, memorise the location of anatomical structures, and quickly identify anatomical structures with 3D methods (Park et al., 2019). Permanently learned information needs less repetition, and time is not lost with constant repetition. It seems possible with the dynamic and interactive environment that 3D models offered to the students may have advanced problem-solving skills and can use what they have learned in practice by realising permanent learning in all areas of educational activities (Ardıç et al., 2017, Park et al., 2019).

Conclusion

In recent years, the number of education method based studies has increased. The comparison of the findings of these studies could be very important in terms of enabling the most effective and efficient ways to be used. According to the results of this study, it can be recommended to expand the number of samples in future studies, re-evaluate the 3D models to be used, and make plans based on the point that permanence tests are effective in determining academic success.

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Conflict of interest

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

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Animal Health, Production and Hygiene

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Research Article

Prevalence of *Cryptosporidium* spp. in Dogs in The Aegean Region

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ABSTRACT

Cryptosporidium spp. is a protozoal parasite that can cause significant gastrointestinal diseases in humans and animals. Those parasites cause infection, especially in young and immunosuppressed animals in many mammals, poultry, reptiles, and humans. *Cryptosporidium spp*. localised in the digestive system, epithelial cells threaten human and animal health with their zoonotic properties. The study's animal material consisted of 200 dogs of different breeds, ages and sexes, including healthy (n=50) and diarrhoea (n=150). Stool samples from the dogs used in the study were stained using the modified Ziehl-Neelsen technique and examined under a microscope. The prevalence of *Cryptosporidium* spp. in faecal samples taken from all dogs was 15.5% and also it was 14% and 16% in healthy and diarrheal dogs, respectively. It was concluded that these results could be used as a reference for future studies on dogs.

Keywords: Cryptosporidium, dogs, prevalence

Ege Bölgesi'ndeki Köpeklerde *Cryptosporidium* spp.'nin Prevalansı

ÖZET

Cryptosporidium spp. insanlarda ve hayvanlarda önemli gastrointestinal hastalıklara sebep olabilen protozoal bir parazittir. İnsan dahil birçok memeli, kanatlı ve sürüngende sindirim sistemi epitel hücrelerine lokalize olan bu parazitler özellikle genç ve immun sistemi baskılanmış hayvanlarda enfeksiyona neden olmaktadır ve zoonotik özelliği ile insan ve hayvan sağlığını tehdit etmektedir. Araştırmanın hayvan materyalini farklı ırk, yaş ve cinsiyette sağlıklı (n=50) ve ishalli (n=150) olmak üzere 200 köpek oluşturdu. Çalışmada kullanılan köpeklerden dışkı örnekleri modifiye Ziehl-Neelsen tekniği ile boyandı ve mikroskopta incelendi. Tüm köpeklerden alınan dışkı örneklerinde *Cryptosporidium* spp. prevalansı %15,5, sağlıklı ve ishalli köpeklerde ise sırasıyla %14 ve %16 idi. Bu sonuçların köpekler üzerinde yapılacak ileri araştırmalar için referans olarak kullanılabileceği sonucuna varıldı.

Anahtar Kelimeler: Kriptosporidyum, köpekler, prevalans

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Introduction

Cryptosporidiosis; is a zoonotic infection caused by *Cryptosporidium* spp., a coccidian protozoan belonging to the class *Apicomplexa*, in many animal species and humans (Wright and Coop, 2007). Compared to other parasites belong in the coccidia family, *Cryptosporidium* spp. shares many similarities in its life cycle (having sexual and asexual forms) but differs from coccidia in some features (Divers and Peek, 2008). In this context, the sporulated form of *Cryptosporidium* oocysts are excreted in the faeces and can cause auto-infection in the animal (Hamnes et al., 2006; O'Handley and Olson, 2006). *Cryptosporidium* spp. and other coccidia parasites differ in their host specificity and size. *Cryptosporidium (C.) parvum* is less host-specific. *Cryptosporidium* spp. is much smaller, and it can not be readily determined by the faecal flotation method (Divers and Peek, 2008). In addition, Cryptosporidium is more resistant to external agents and has more distinct areas of localization regions in cells than other coccidia (Hamnes et al., 2007).

Cryptosporidium canis is a genotype of *Cryptosporidium spp*. that affects dogs (Fayer et al., 2001). The first report of *Cryptosporidium* spp. was published in England in 1983 (Wilson et al., 1983). Since then, several studies have been documented on the occurrence, prevalence and risk factors of cryptosporidiosis in dogs (Bajer et al., 2012). *C. canis* and its subgenotypes have been reported worldwide in dogs, foxes, coyotes, and humans (Fayer et al., 2001; Lucio-Forster et al., 2010; Elwin et al., 2012). It is generally species-specific, dogs are commonly infected with *C. canis*, and it has also been reported that they are infected with *C. parvum* (Scorza and Tangtrongsup, 2010; FitzGerald et al., 2011; Scorza et al., 2014). *C. canis* is often subclinical in dogs, and these dogs excrete oocysts without showing any clinical findings (Lindsay and Zajac, 2004; Scorza and Tangtrongsup, 2010;). Epidemiological studies in dogs with *Cryptosporidium spp*. have shown that disease prevalence varies by the geographic region, and there is no correlation between age groups or living conditions. Previous studies have shown that young and adult dogs can excretion of oocysts with their faeces (Huber et al., 2005). Chronic or intermittent diarrhoea, anorexia and wasting are the most common clinical findings in symptomatic dogs. Dogs with clinical findings are usually young animals (Lucio et al., 2016). The mechanism of diarrhoea, malabsorption and attenuation induced by *Cryptosporidium* is not fully understood (Lindsay and Zajac, 2004). Oocyst excretion has been shown to persist over a long period of time (more than 3-5 months) in experimentally infected animals. (Asahi et al., 1991). Cryptosporidiosis in dogs is transmitted directly or indirectly by the fecal-oral route. It occurs directly during coprophagy and indirectly through contaminated food and water (Baldursson and Karanis, 2011).

There are few studies on the prevalence of *Cryptosporidium* spp. in dogs. *Cryptosporidium spp*. prevalence was found to be 8% (5/62) in animal shelters and 10% (8/78) in veterinary clinics in Canada (Uehlinger et al., 2013). Epidemiological studies have shown that variation in prevalence depends primarily on the methods used and then on geographic location. It was reported as 0% in Austria (Bugg et al., 1999) and 5% in Argentina when the flotation method was used (Fontanarrosa et al., 2006). Percentages from the United Kingdom (Batchelor et al., 2008) and the Netherlands (Overgaauw et al., 2009) were reported as 0.6 and 8.7, respectively when the staining method was used. Data from prevalence studies with ELISA; Italy 1.7%, Canada 7.4%, Germany 23% (Bauer et al., 2004; Shukla et al., 2006; Rinaldi et al., 2008). A similar prevalence was 44% with the immunofluorescence technique in Norway (Hamnes et al., 2007). This high prevalence may be related to the commercial kit (Titilincu et al., 2010). It has been reported that the specificity and sensitivity of the ELISA kit are lower (94% and 71%, respectively) than immunofluorescence microscopy in *Cryptosporidium* spp. (Rimhanen-Finne et al., 2007). Parasites of dogs are not only crucial for animals but also for humans because of the zoonotic potential of some species and the need for frequent monitoring. While most human infections are caused by *C. parvum* and *C. hominis*, a certain percentage are caused by *C. canis* (Ryan et al., 2014). Several pathogens can cause mild intestinal diarrhoea in dogs. Therefore, native slide examination and faecal flotation are initially applied to the diagnostic approach (Mundim et al., 2007). Immunological methods are also used in the identification of the parasite. Direct Immunofluorescence Technique (DFA), Latex Agglutination Reaction, Reverse Passive Haemagglutination, Immunochromatography, Indirect Fluorescent Antibody Test (IFA), Enzyme-Linked Immunosorbent Antibody Test (ELISA) and rapid diagnosis kits are commercially available for the immunological investigation of *Cryptosporidium* in the stool (Fayer et al., 2001; Babac, 2014).

While there are several studies on the prevalence of *Cryptosporidium* spp. in humans and some animal species in our country, there are no studies on the prevalence of *Cryptosporidium spp*. in dogs. This study aimed to determine the prevalence of *Cryptosporidium spp*., which has a zoonotic feature, in dogs in the Aegean region.

Material and Methods

The research was started with Aydın Adnan Menderes University Veterinary Faculty Ethics Committee Ethics Committee (Date: 22.08.2017, Number: 64583101/2017/081). All stool samples were collected voluntarily by informing the owner. The material of the study was collected from dogs brought to Aydın Adnan Menderes University Veterinary Faculty Research and Application Hospital, polyclinics, some private veterinary clinics and animal hospitals in Aydın, İzmir, Manisa, Denizli and Muğla provinces between March 2018 and March 2019. The study included 200 dogs of different breeds, ages and sexes (50 healthy and 150 with diarrhoea). Stool samples were taken into sterile storage containers. After flotation in sucrose solution, the stool was stained using the modified Ziehl-Neelsen technique (Scorza and Lappin, 2012). The samples were prepared from the dyed materials by the smear method. They were examined

under the microscope at 100x magnification with a drop of immersion oil. This examination was performed to determine the status of the presence or absence status of *Cryptosporidium* spp. oocysts. They were defined as round structures with a diameter of 4-6 µm, which were heterogeneously stained in bright pink-red colour on a blue background, and most of them had black irregular granules.

The data obtained from the study were evaluated in the SPSS package program (SPSS© 22.0, IBM, Armonk, NY). The Shapiro-Wilk test determined whether it showed a normal distribution. Analysis of proportional data was evaluated by chi-square analysis. The level of error (*P*) was taken as 0.05.

Results

The prevalence of *Cryptosporidium* spp. in stool samples taken from dogs was 15.5% (31/200). At the same time, 24 samples out of 150 dogs (16%) with diarrhoea and 7 samples from 50 healthy dogs(14%) were positive for *Cryptosporidium* spp. (Table 1).

Table 1. Distribution of *Cryptosporidium* spp. in healthy and diarrheal dogs (Number, %).

		Samples		Total
		Positive	Negative	
Condition	Healthy	7 (14%)	43 (86%)	50 (100%)
	Diarrheal	24 (16%)	126 (84%)	150 (100%)
Total		31 (15.5%)	169 (84.5%)	200 (100%)

X^2=0.115 P<0.05

When the rate of Cryptosporidium positivity according to gender was examined, it was determined at 16.4% in male dogs and 14.6% in female dogs (Table 2).

Table 2. *Cryptosporidium* spp. incidence rates by gender (Number, %).

		Samples		Total
		Positive	Negative	
Sex	Male	15 (16.4%)	76 (83.5%)	91 (100%)
	Female	16 (14.6%)	93 (85.3%)	109 (100%)
Total		31 (15.5%)	169 (84.5%)	200 (100%)

X^2=1.802 P<0.05

Cryptosporidium positive rate by age group; was seen at 11.3% in dogs aged 0-6 months, 24.1% in dogs aged 7-24 months, and 14.3% in dogs 25 months and older (Table 3).

14% positive in dogs with solid stool, 20.3% positive in dogs with pasty stool, 14.3% positive in dogs with liquid stool; this rate was found to be 5.6% in dogs with hemorrhagic stools when evaluated by stool consistency groups (Table 4).

Discussion

Cryptosporidium, which is localised to the epithelial cells of the digestive system in many mammals, including humans, poultry and reptiles, is a protozoal parasite that can cause significant gastrointestinal diseases (Mundim et al., 2007). These parasites cause infection in immunocompromised patients. In addition to the chemical and physical characteristics of the agent in the spread of the parasite, the presence of many hosts, the ease of spread of the parasite and its ability to infect a small number of oocysts increase its importance to animal and human health (Scorza and Lappin, 2012).

It has been reported that the parasite is naturally found in the flora. *Cryptosporidium* spp. infections are generally observed in asymptomatic form in dogs (Hall and Day, 2017). *Cryptosporidium* spp. infects the organism based on the developmental stages in the epitelium of the middle and last duodenal mucosa and the epithelial parts of the head and middle jejunal mucosa (Wilson et al., 1983; Greene et al., 1990). It causes villus atrophy and inflammation in the affected area (Koudela and Jiri, 1997). As a result, malabsorption and maldigestion develop and cause various symptoms in the organism. It is reported that the affected area in the organism differs depending on the *Cryptosporidium* species (Plutzer and Karanis, 2009). Although four different *Cryptosporidium* species have been identified in dogs, *C. canis, C. parvum* and C*. meleagridis* have been reported to settle in the small intestine and *C. muris* in the stomach (Cuia et al., 2018). It has been reported that the affected area in the organism differs according to Cryptosporidium species (Plutzer and Karanis, 2009). In this study, *Cryptosporidium* spp. was determined in symptomatic and asymptomatic dogs without species identification.

Laboratory diagnosis of cryptosporidiosis involves microscopic, serologic, and molecular methods. Diagnosis can be supported by direct microscopic examination using various staining methods, immunofluorescence methods, ELISA, and PCR methods. IFA is an essential but expensive technique for diagnosis in the early stages of the disease. Specific anti-*Cryptosporidium* spp. IgG and IgM can be detected by the ELISA method. Recently, PCR techniques have been developed for diagnostic purposes. However, their use in diagnosis is limited because they are expensive. The use of more than one

Table 3. *Cryptosporidium* spp. incidence rates by age groups (Number, %).

		Samples		Total
		Positive	Negative	
Age Groups	0- 6 months	11 (13.3%)	86 (88.7%)	97 (100%)
	7-24 months	13 (24.1%)	41 (75.9%)	54 (100%)
	≤25 month	7 (14.3%)	42 (85.7%)	49 (100%)
Total		31 (15.5%)	169 (84.5%)	200 (100%)

X^2= 0.113 P<0.05

method in combination with microscopic methods for diagnosis increases the reliability of the results (Bennett et al., 1985). It has been reported that many different prevalence studies have been performed in dogs using other methods (Lindsay and Zajac, 2004). *Cryptosporidium spp*. oocysts were determined in stool samples collected in this study, as in many other studies, using the Ziehl-Neelsen staining technique.

Although it has been reported that cases of cryptosporidiosis in dogs generally occur in young individuals, epidemiological studies have reported that there is no relationship between age and infection in *Cryptosporidium* spp. infections (Moreira et al., 2018). However, some authors noted that the infection rate is higher in young dogs (Pivoto et al., 2013; Olabanji et al., 2016), while some researchers referred to adult dogs (Bresciani et al., 2008). While Pivoto et al. (2013) argue that there is no relationship between age and infection, they report that the percentage of oocysts (25%) in animals under one year old is slightly higher than the percentage of animals older than one year (23.2%). The researchers emphasise that age is not a risk factor. The prevalence of infection is higher in dogs three to six months of age. In contrast, Bresciani et al. (2008) reported a higher prevalence of infection in animals one to four years of age. Thompson et al. (2005) note that although infections are recurrent in adult animals, disease frequency is higher in young animals. Noordeen et al. (2001) and Bajer et al. (2012) emphasise that prevalence is higher in young animals other than dogs. In this study, in agreement with many authors, although no correlation was found between Cryptosporidium infection and age, the highest prevalence (24.1%) in the age group between 7-24 months was in agreement with the results of Gbemisola et al.(2016).

Table 4. *Cryptosporidium* spp. incidence rates according to stool consistency groups (Number, %).

		Samples		Totally
		Positive	Negative	
Stool Consistency	Solid	7 (14%)	43 (86%)	50 (100%)
	Pasty	14 (20.3%)	55 (79.7%)	69 (100%)
	Fluid	9 (14.3%)	54 (85.7)	63 (100%)
	Hemorrhagic	1 (5.6%)	17 (94.4%)	18 (100%)
Totally		31 (15.5%)	169 (84.5%)	200 (100%)

X^2=0.463 P<0.05

Although sex has been reported to play no role in *Cryptosporidium* spp. infection (Mundim et al., 2007), it is said to be relatively more common in female dogs than in males. Zelalem and Addis (2012) reported that the prevalence of *Cryptosporidium* was higher in males (79.2%) than in females (76.8%). It has been reported that the higher incidence in females may be related to decreased immunity during certain periods due to physiological cycle.

When evaluating the sex distribution of infected animals in this study, the disease incidence was found to be 16.4% in males and 14.6% in females. Previous studies reported that there was no sex predisposition in *Cryptosporidium* spp. parasite infections (Mundim et.al., 2007). There was no association between Cryptosporidium prevalence and the sex of dogs, suggesting that both sexes may have an equal chance of becoming infected when exposed to an infected or contaminated substance.

The prevalence of *Cryptosporidium* infection is reported to be higher in pure breeds. Different breeds of dogs were used in this study. For this reason, it was assumed that it would not give a healthy result and was not involved in the statistical analysis.

The prevalence of Cryptosporidium in dogs is range from 0% and 44.8% in many studies conducted worldwide (Lindsay and Zajac, 2004). The prevalence depends on the determination method, geographic location, care, feeding, environmental conditions, and cleaning-disinfection (Cirak and Bauer, 2004; Shukla et al., 2006). In the prevalence study conducted by ELISA, the prevalence was found to be 1.7% in Italy, 7.4% in Canada, and 23% in Germany (Cirak and Bauer, 2004; Shukla et al., 2006). A similar prevalence was 44% by immunofluorescence technique in Norway (Hamnes et al., 2007).

Reported rates on direct microscopic examination of faecal samples using various staining methods in dogs range from 2% to 17% in the USA; 8.8% in Brazil; 2.2% in Argentina; 6.3% in Spain; 9.7% in Korea, and 11% in Australia (Scorza and Tangtrongsup, 2010). The prevalence of *Cryptosporidium* spp. was found to be 8% (5/62) in shelters and 10% (8/78) in veterinary clinics in Canada (Uehlinger et al., 2013). There is no study on the prevalence of *Cryptosporidium* spp. in our country. In this study, the prevalence of *Cryptosporidium* spp. in dogs in the Aegean region was 15.5% (31/200).

Conclusion

According to the consistency of the stool, classification was defined as healthy in animals with solid stool status. As a result of the diagnostic applications, it was determined that the incidence of *Cryptosporidium* was 14% in animals classified as healthy and 16% in animals with diarrhoea. This situation supports that the agent can widely be asymptomatic. It was determined that age, gender and faeces consistency were not crucial in the prevalence of *Cryptosporidium.* At the same time, it is thought that taking these factors into account can be used as a reference for future studies on dogs.

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This study was summarised by the first author's Master Thesis.

Conflict of interest

The authors declare that they have no conflict of interest.

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Case Report

Traumatic Membranous Urethral Rupture in a Foal: A Case Report

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ABSTRACT

Urethral ruptures can be a life-threatening emergency that may lead to fatal uroperitoneum. This case report aimed to describe the clinical findings and treatment results of a traumatic membranous urethral rupture in a 15-day-old Ambling foal. The foal had been wounded by a wild animal 9 days before, and despite treatment, the foal's condition worsened, the owner brought the animal to Faculty of Veterinary Medicine Research and Practice Animal Hospital. On physical examination several wounds in the pubic and inguinal regions were identified, some of which were complicated by a fistula. Vital parameters of the foal were not in reference ranges. In laboratory findings, lymphopenia, eosinopenia, mild anemia, and decreased partial oxygen pressure with acidic blood pH were observed. Sutures were applied surgically under general anesthesia to the urethral tear and wound care was provided. On the fifth day after surgery, the owner called the hospital to report that the foal was constipated and had signs of colic. Per rectal olive oil usage was recommended, and surgery was re-planned for the following day. Despite cardiac resuscitation and emergency treatments, the foal died during the operation due to cardiac arrest. In conclusion, gastrointestinal outcomes of surgery and anesthesia must be considered.

Keywords: Foal, horse, urethra, rupture

Bir Tayda Travmatik Membranöz Üretra Rupturu: Vaka Raporu

ÖZET

Üretral rupturlar üroperitona yol açabilen hayatı tehdit eden bir acil durum olabilmektedir. Bu olgu sunumu, 15 günlük bir rahvan ırkı tayda travmatik membranöz üretra rupturunun klinik bulguları ve tedavi sonuçları hakkında bilgi vermeyi amaçlamıştır. Dokuz gün önce vahşi bir hayvan tarafından yaralanan tay, uygulanan tedaviye rağmen durumu ağırlaşınca sahipleri tarafından Veteriner Fakültesi Araştırma ve Uygulama Hayvan Hastanesi'ne getirildi. Yapılan fiziki muayenede tayın kasık ve inguinal bölgelerinde bazıları fistülle komplike olmuş birkaç yara tespit edildi. Tayın rektal vücut sıcaklığı, kalp atım hızı, solunum frekansı ve kapiller dolum zamanının düşük olduğu gözlendi. Laboratuvar bulgularında lenfopeni, eozinopeni, hafif anemi, asidik kan pH'sı ile parsiyel oksijen basıncının düştüğü gözlendi. Üretra yırtığına dikiş atıldı ve açık yaralara yara bakımı yapıldı. Ameliyattan sonraki beşinci gün, sahibi hastaneyi arayarak tayın kabız olduğunu ve kolik belirtileri olduğunu bildirdi. Rektal zeytinyağı kullanımı önerildi ve ertesi gün için ameliyata karar verildi. Kardiyak resüsitasyon ve acil tedavilere rağmen tay, operasyon sırasında kalp durması nedeniyle kurtarılamadı. Sonuç olarak, sindirimi ilgilendirmeyen cerrahi operasyonlarda bile cerrahi ve anestezik prosedürlerin gastrointestinal kanal üzerindeki sonuçları göz önünde bulundurulmalıdır.

Anahtar kelimeler: Tay, at, uretra, ruptur

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Introduction

Ruptures in the lower urinary tract may have a life-threatening emergency that may result in uroperitoneum. Urinary tract rupture is less common in horses (Schott and Woodie, 2012). A urethral laceration is most encountered in horses in the penile or extra-pelvic urethra due to its superficial location and vulnerability to trauma (Schott and Woodie, 2012). Uroperitoneum (Richardson and Kohn, 1983; Adams et al., 1988; Lavoie and Harnagel, 1988; Kablack et al., 2000; Dunkel et al., 2005), urachal abscess and ruptures (Hyman et al., 2002), and congenital anomalies of ureters has been widely reported in previous studies (Robertson et al., 1983; Divers et al., 1988; Cutler et al., 1997; Jean et al., 1998; Morisset et al., 2002). Despite reports of urethral and bladder rupture in foals (Castagnetti et al., 2010; Oreff et al., 2015), little is known about a membranous urethral rupture in newborn foals. This case report describes the surgical approach and therapy used to treat a foal with urethral rupture.

Case History and Clinical Findings

Fifteen days old, an Ambling male foal weighing 30 kg was brought to the Aydın Adnan Menderes University, Faculty of Veterinary Medicine Research and Practice Animal Hospital because of a wild animal attack. The foal had a normal delivery and was healthy until the day of the wild animal attack occurred based on the claim of the owner. A freelancer veterinarian was applied wound care (10% povidone-iodine emulsified gauze dressing), flunixin meglumine (50 mg/kg, i. m., q. d.), and penicillin G sodium (20,000 IU/kg, i. v., q. 6h) injections and referred the foal to the hospital after 9 days. The foal was depressed and weak in the physical examination (Figure 1a). Rectal temperature, heart rate, respiration rate, and capillary refill time were low (28 bpm, 37.3°C, 10 breaths/min, and 4 seconds, respectively). Several wounds on the pubic and inguinal regions were found (Figures 1b-d). Two of the wounds had fistulae, and the discharge was slightly yellow. One of the wounds measured over 12 cm horizontally, parallel to the penis, and was filled with urine. Urine discharge was observed from one of these wounds. Jugular venous and arterial blood samples were obtained. On complete blood count and blood gasses analysis lymphopenia, eosinopenia, decreased partial oxygen pressure, decreased blood pH and mild anemia was detected.

Anesthesia was induced with xylazine HCl (0.6 mg/kg, XylazinBio® %2, Bioveta PLC, Ivanovice na Hane, Czech Republic) and ketamine HCl (1.5 mg/kg, Ketasol® %10, Richter Pharma Ag, Wels, Austria) i. v. and maintained with 1.5% isoflurane (Isoflurane USP®, Adeka Ilac, Istanbul, Turkey) in 100% oxygen as a standard anesthesia protocol of the hospital. The foal was placed in a dorsal recumbent position, and the ventral aspect of the abdominal wall was clipped and prepared aseptically for surgery (Figure 1c). A 5-centimeter-long urethrotomy was performed near the wound's medial margin (Figure 1d). A sterile 14 French Foley catheter was placed through the urethra and secured in place with a single purse-string suture for ensuring urine drainage (Figure 1e). The urethral defect, subcutaneous tissues, and skin were closed separately with 2-0 USP monofilament Polyglecaprone 25 (Monocryl®, Ethicon Inc., Raritan, New Jersey, United States) in a simple continuous partial-thickness suture (Figure 1f-n). Sterile gauzes were inserted as a drain for fistulated open wounds. Procaine penicillin (8 mg/kg, q. d., i. m.) and streptomycin (10 mg/kg, q. d., i. m.) injections and local ether iodoform (q. d.) were prescribed for postoperative 7 consecutive days. During anesthesia recovery, the foal's vital signs were normal, and the animal was discharged 4 hours after the surgery. Flunixin meglumine (50 mg/kg, i. m., q. d.) was prescribed post-operatively. The owner called the hospital on the evening of the fifth day after the operation to report that the animal had not defecated for five days and was showing signs of colic (biting and kicking its flank, frequently looking at its side, and poor eating behavior). Rectal use of olive oil was recommended to the owner for constipation. The same night, the owner called the hospital again and reported urine leakage from the treated wound areas;

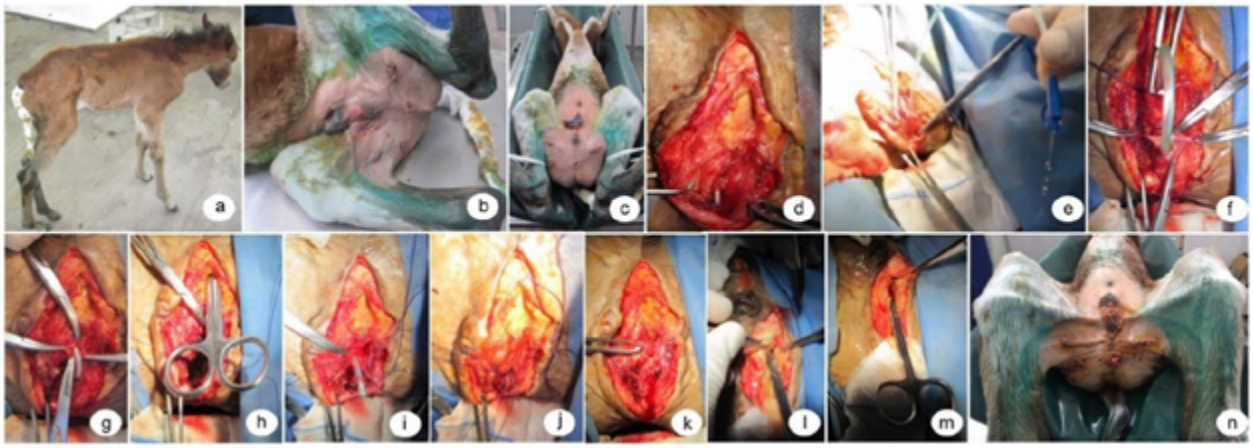

Figure 1. The foal's physical appearance prior to surgery pre-operative preparation and findings, intraoperative interventions, and post-operative photograph of the operation site.
a= prior to surgery; b and c= pre-operative preparation and findings; d, e, f, g, h, I, j, k, l, and m= intraoperative interventions; n= post-operative photographs.

the foal was planned for reoperation the next morning due to wound dehiscence. Vital signs of the foal (rectal temperature, heart rate, femoral pulse, capillary refill time, and respiratory rate) were within reference ranges. Mild anemia and increased white blood cell count were detected. The animal was anesthetized according to the same procedure. Cardiac arrest occurred in operation. The foal couldn't be saved despite cardiac resuscitation and emergency interventions.

Discussion

This case report describes a membranous urethral rupture that resulted in the animal's death due to a postoperative complication.

Urethral rupture is rare in humans, small animals, and horses (Anderson et al., 2006; Meige et al., 2008; Schott and Woodie, 2012). Furthermore, the solitary indicative clinical symptom of urethral rupture is sometimes noticeable soft tissue swelling in the surrounding tissue, which can be difficult to diagnose (Schott and Woodie, 2012). Even though 9 days had passed since the wild animal attack, the diagnosis of urethral tear was made by visual observation of urine discharge from wound sites, aside from the fact that urine retention was found in the abdomen, complete blood count did not reveal azotemia, and none of the clinical signs related to azotemia was noted. Urine leakage from distal urethral areas tends to have fistulae, and the animal may have milder azotemia-related systemic symptoms (Boothe, 2000). In the present case report, the existence of wounds in the animal's caudal abdominal and inguinal regions, as well as loss of preputial integrity, suggested the presence of urethral rupture, and the diagnosis was made by observing urine leakage from the wounds.

An indwelling urinary catheter and therapy for fluid-electrolyte and acid-base abnormalities can be used as a conservative treatment for the urethral tear (Boothe, 2000; Schott and Woodie, 2012). For more severe urethral injuries, surgical management is required, which requires gentle manipulation of the surgical site, ensuring blood circulation, and precise tissue apposition (Boothe, 2000; Schott and Woodie, 2012). Urine contamination in the periurethral tissues causes periurethral fibrosis and late wound healing (Anderson et al., 2006). When urine is rerouted away from the surgical site or the site of injury, the uroepithelium can bridge a defect in 3–21 days (Anson, 1987; Holt, 1989). The urinary tract diversion approach was highlighted in a study on dogs and cats (Anderson et al., 2006). Castagnetti et al. (2010) reported that urethra and bladder rupture in a foal was repaired by suturing the wounds and tears. Also, they reported wound dehiscence as a postoperative complication (Castagnetti et al., 2010). Urine diversion by laparoscopic aided tube cystotomy was claimed to be a successful intervention in another similar report of urethral rupture in a foal after two attempts with no success to repair the tear by primary closure (Oreff et al., 2015). In the present report, the primary closure approach was used as a surgical attempt. However, the tear was dehiscence after 5 days of surgical intervention. The presence of constipation could be the consequence of dehiscence leading to increased pressure on the skin by colic. According to previous research, there is an intermediate clinical phase in horses after surgery unrelated to the gastrointestinal tract, which is characterized by decreased fecal output before observable signs of colic (Little et al., 2001).

In conclusion, even for urethral ruptures, constipation and colic may be postoperative complications of surgical intervention in foals. Postoperatively, gastrointestinal features should be closely examined, and fecal softeners may be recommended. Also, anesthesia protocol, pain management, and special diets may be considered.

Acknowledgments

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Case Report

Anoplasty Surgery in a Cat with Type I Atresia Ani

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ABSTRACT

Atresia ani (AA) is a congenital defect that occurs when the anal membrane is not perforated and becomes permanent. However, it is worthwhile to note that it is not very common in cats. This case report consists of a 2-month-old male Persian kitten with Type I AA, who was brought to Aydın Adnan Menderes University Faculty of Veterinary Medicine Research and Practice Animal Hospital, Surgery Department with the complaint of defecation from a very narrow opening since birth. In the clinical examination of the patient, abdominal distention and swelling around the anus were detected. The patient was constantly taking a defecation position and had difficulty defecating. Contrast radiography was taken with the retrograde method, and the obstruction in the anus was detected resulting in the decision of performing an operation. The patient was placed on the table in the prone position and the narrowed part of the rectum was treated by resecting it with 360° anoplasty. Having lowered the rectal mucosa distally, the mucosa and skin were brought together, and then sutured with synthetic non-absorbable monofilament 4/0 suture material. Extra care was taken to preserve the external anal sphincter and anal sacs during dissection. The aim of surgical intervention is to maintain anorectal continuity as well as preserving the external anal sphincter and colon function. Surgical treatment should be promptly performed before chronic and prolonged distention. In the presented case, even though the kitten is 2 months old, successful results were obtained with surgical treatment after diagnosis.

Keywords: Anoplasty, anorectal malformation, cat, congenital anomaly, imperforated

Atresia Ani Tip I Bulunan Yavru Kedide Anoplasti Operasyonu

ÖZET

Atresia ani (AA), anal membranın yıkımlanmayıp kalıcı hale gelmesiyle oluşan ve kedilerde nadir karşılaşılan doğmasal bir anomalidir. Bu olgu raporu, doğumundan itibaren çok dar bir açıklıktan zor dışkılama şikayetiyle Aydın Adnan Menderes Universiesi Veteriner Fakültesi Hayvan Hastanesi, Cerrahi Anabilim Dalına getirilen ve Atresia Ani Tip I teşhisi koyulan 2 aylık erkek İran kedisinde uygulanan anoplasti operasyonunu içermektedir. Hastanın klinik muayenesinde abdominal gerginlik ve anüs çevresinde şişkinlik saptandı. Hasta sürekli olarak dışkılama pozisyonu almasına rağmen dışkılama güçlüğü çekmekteydi. Retrograd yöntemle kontrast radyografi alınarak anüsteki daralma saptandı ve operatif tedaviye karar verildi. Hasta yüz üstü pozisyonda masaya yerleştirildi ve rektumun daralan kısmı 360° anoplasti ile rezeke edilerek tedavi edildi. Daha sonra, rektal mukoza distale doğru indirilip mukoza ve deri karşı karşıya getirilerek sentetik emilemeyen 4/0 monofilament dikiş materyaliyle dikildi. Diseksiyon sırasında eksternal anaf sfinkter ve anal keselerin korumasına dikkat edildi. Cerrahi müdahalenin amacı anorektal devamlılığı sağlamak ve eksternal anal sfinkter ile kolon fonksiyonunu korumaktır. Kronik ve uzun süreli şişkinlik ile ilişkili kolonik atoni ya da megakolon ortaya çıkmadan önce cerrahi tedavinin yapılması gerekir. Sunulan olguda da her ne kadar 2 aylık yaşa gelmiş olsa da teşhis sonrası hızlıca cerrahi tedavi ile başarılı sonuç alınmıştır.

Anahtar kelimeler: Anoplasti, anorektal malformasyon, imperfore, konjenital anomali

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Introduction

Congenital anorectal deformities are rarely encountered in small animals (Fossum, 2013; Pamuk et al., 2009). AA is a congenital defect in the anorectum that results in closure of the anal canal and/or abnormal routing of feces (Papazoglou & Ellison, 2012). It develops due to incomplete separation of the urorectal fold from the primitive cloaca or inability to perforate the anal membrane after anus formation (Vianna & Tobias, 2005) including congenital anal stenosis (Type I. Four types of AA have been described in small animals. In Type I AA there is a congenital stenosis of the anus, whereas in Type II AA; the anal membrane is present and ends as a blind sac just cranial to the anus, with the rectum not punctured. In Type III AA; the anus is closed and the blunt end of the rectum ends more cranially than Type II. Lastly, in Type IV A; the anus and the last section of the rectum are developed normally, but the rectum ends in the pelvic canal in the form of a blind sac. Rectovaginal fistula (RVF) formation may occur in female cats in Type II AA (also rarely in Type III AA) (Figure 1) (Kurt & Turan, 2021). In this case report, the treatment with anoplasty surgery in a kitten with Type I AA will be described.

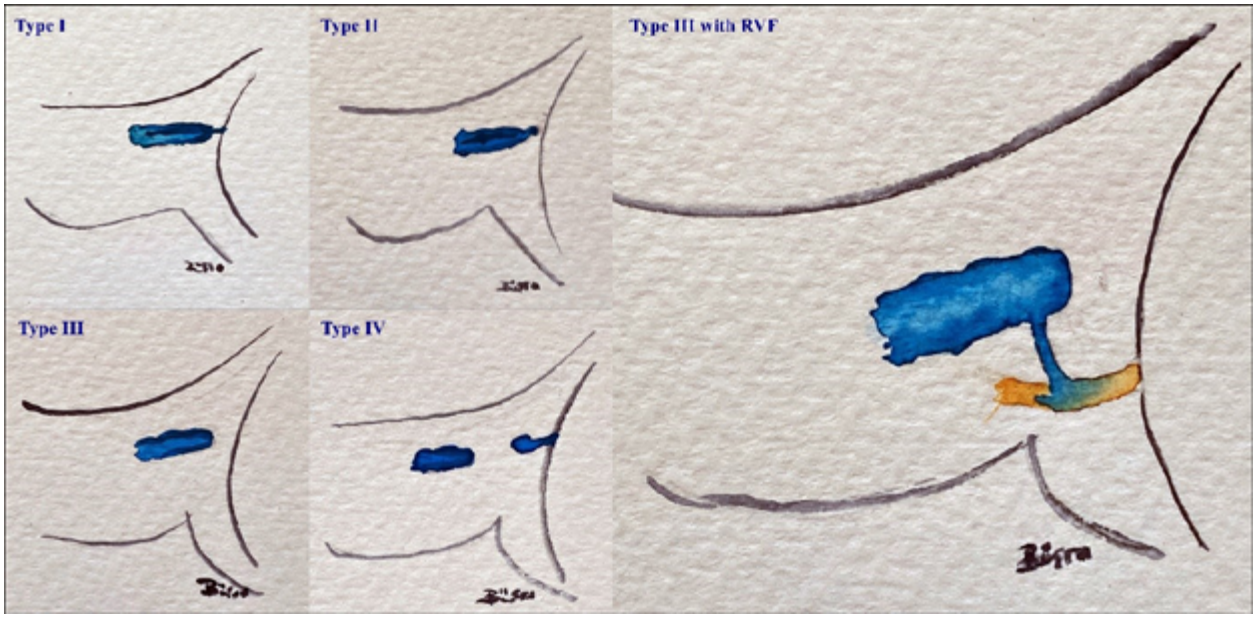

Figure 1. Types of AA, RVF: Rectovaginal fistula (Kurt & Turan, 2021).

Case History and Clinical Findings

The study consists of a 2-month-old Persian kitten with a body weight of 1 kilogram who was brought to Aydın Adnan Menderes University Faculty of Veterinary Medicine Research and Practice Animal Hospital, Surgery Department with the complaint of defecation through a very narrow opening since birth. In the clinical examination of the patient, abdominal distention, swelling around the anus, and a vulva-like cleft between the anus and the penis were detected (Figure 2). The patient took a defecation position and had difficulty defecating. Dense stool was detected in the intestines at the laterolateral oriented abdominal X-ray (Figure 3). Anal stenosis was detected by retrograde contrast radiography and surgical intervention was decided (Figure 4).

Figure 2. Vulva-like cleft (arrow) between anus and penis.

Cephalosporin (Sef 250 mg/5ml oral suspansiyon®, 20 mg/kg, oral, twice a day, Gensenta İlaç Sanayi ve Ticaret A.Ş., Turkey) and nutritional support (Viyo Recuperation Cat®, 30 ml, daily, Vito International, Belgium) was prescribed to be administered for the patient 3 days before the operation. The patient was general anesthetized with xylasin (Xylazin Bio 2%®, 1 mg/kg, intramuscularly, Bioveta PLC, Czech Republic) and ketamine (Ketasol 10%®, 11 mg/kg, intramuscular, Richter Pharma AG, Austria). The patient was placed in the sternal position, and then the narrowed part of the rectum was resected with 360° anoplasty. The anal opening was widened by suturing the rectal mucosa with simple separate sutures and a 4-0 monofilament polypropylene suture (Atramat®, Mexico) to the mucosa (Figures 5 and 6). Cephalosporin (Sef 250 mg/5ml oral suspansiyon®, 20 mg/kg, oral, twice a day, Gensenta

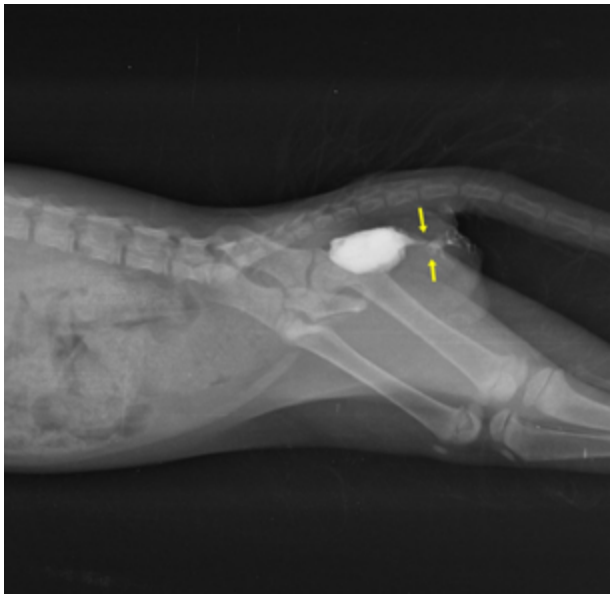

Figure 3. Fecal impaction on radiography.

Figure 4. Indirect radiography, anal stenosis (arrow).

İlaç Sanayi ve Ticaret A.Ş., Turkey) was used in the postoperative 5-day period. Nutritional support (Viyo Recuperation Cat®, Vito International, Belgium) and kitten wet food were given as postoperative nutrition (N&D Prime Chicken & Pomegranate Kitten®, Farmina, Italy).

No problem was detected about defecation in the postoperative 25th day control.

Discussion

AA requires immediate treatment. As AA usually goes unnoticed, kittens die soon after birth. It can be diagnosed by checking the defecation of kittens after birth or in suspicious cases by detecting fecal accumulation inside the intestines by radiography.

In Type I AA, the owner of the patient may state in her or his anamnesis that there is difficulty in defecation and straining after the kitten has been weaning. Applications such as special diets, laxatives, and enemas fail. On physical examination, there is tenderness and an anal reflex. Definitive diagnosis can be achieved by radiography (Prassinos et al., 2003). Abdominal radiography can be used to evaluate the degree of colonic dilatation and rectum. Studies have reported that the diagnosis of Type I AA can also be maintained by ultrasonography and the length of the narrowed region can be determined by this method. For evaluating and conducting anatomic typing of AA in small animals, computed tomography and magnetic resonance may also be useful (Ellison & Papazoglou, 2012). Based on clinical examination and radiography findings, the patient was diagnosed with Type I AA. The diagnosis was confirmed by surgery.

The prognosis of Type II and Type III AAs are worse than

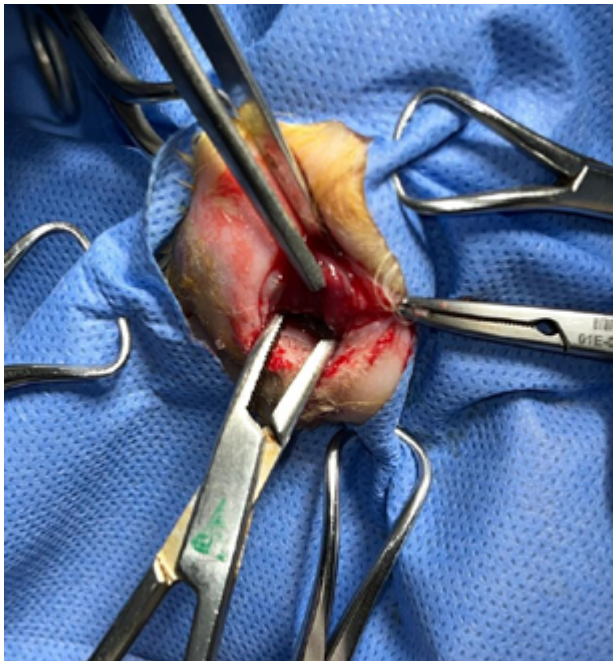

Figure 5. Widening of the anal opening.

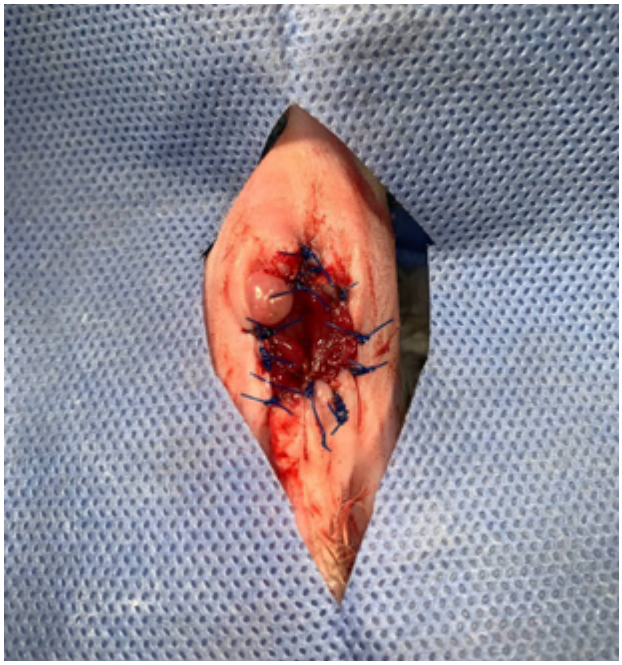

Figure 6. Postoperative anal opening.

in Type I since the colonic dilatation develops (Prassinos et al., 2003). In Type I and Type III AA cases, there is no defecation so surgical treatment is required (Ellison & Papazoglou, 2012). In this study, the kitten survived because faecal output was achieved through a narrow opening. Enema was applied in a private clinic for a long time, but it could not be treated because the opening was narrow and it was transferred to Aydın Adnan Menderes University Faculty of Veterinary Medicine Research and Practice Animal Hospital, Surgery Department. Studies report that deaths can be prevented in cases of atresia ani by early diagnosis and surgical treatment (Aslan et al., 2009). The goal of the treatment is to maintain anorectal continuity, preserve the external anal sphincter and colon function. Surgical treatment should be promptly performed before colonic atony or megacolon occurs, associated with the chronic and prolonged distention (Prassinos et al., 2003). No sign of megacolon was detected in the indirect radiographs.

There is not much literature about the treatment of Type I AA in cats. In Type I AA, the stricture can be treated by anoplasty or balloon dilatation (Papazoglou & Ellison, 2012). In the presented study, successful treatment was achieved with the anoplasty operation. As postoperative nutritional support; Viyo Recurperation® (30 ml daily, Viyo Recuperation Cat, Viyo International, Belgium) was prescribed, which is rich in vitamins, minerals, amino acids, fatty acids and also contains glutamine, arginine, taurine and omega-3 fatty acids.

Conclusion

Cases of atresia ani are often overlooked due to misdiagnosis, delayed treatment, or lack of knowledge about treatment, and patients die or are euthanized. Therefore, few cases are reported in the literature. In this case, diagnosis and surgical correction in Type I AA were successful.

Conflict of Interests

The authors declare that they have no conflicts of interests.

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Review

Roles of Probiotics in Animal Health

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ABSTRACT

Probiotics are defined as live microorganisms consumed by humans and animals that affect the intestinal microflora qualitatively or quantitatively or trigger the beneficial effects of the immune system. The discovery of probiotics and the beginning of studies date back to the end of the 19th century. Afterwards, these studies continue on the microorganisms used as probiotics, selection criteria and probiotic microorganisms in the animal microbiota. Today's probiotics are used as immune system modulation and protection against pathogenic microorganisms in veterinary medicine. In recent studies against gastrointestinal system disorders in cats, dogs and poultry and on the immune system before or after treatment, probiotic applications have been found to be successful in ruminants, especially in mastitis cases. Due to important problems caused by the use of antibiotics in animal breeding, such as the increase in populations of antibiotic resistant bacteria, it seems possible to use the latest probiotic applications as an alternative to antibiotics, especially for prophylaxis. In this review, the effectiveness of probiotic microorganisms on the basis of diseases and their effects on the immune system are discussed together with current studies.

Keywords: Animal health, imuune system, probiotic

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Probiyotiklerin Hayvan Sağlığındaki Rolleri

ÖZET

Probiyotikler kalitatif veya kantitatif olarak bağırsak mikroflorasına etki eden ya da immun sistemin faydalı etkilerini tetikleyen, insanlar ile hayvanların tükettiği canlı mikroorganizmalar olarak tanımlanmaktadır. Probiyotiklerin keşfi ve çalışmaların başlaması 19. yüzyılın sonlarına dayanmaktadır. Devamında probiyotik olarak kullanılan mikroorganizmalar, seçim kriterleri ve hayvan mikrobiyotasında bulunan probiyotik mikroorganizmalar ile ilgili araştırmalar devam etmektedir. Günümüz veteriner hekimliğinde probiyotikler, immun sistem modülasyonu ve patojen mikroorganizmalara karşı koruyucu olarak kullanılmaktadır. Kedi, köpek, kanatlılarda gastrointestinal sistem rahatsızlıklarına karşı ve tedavi öncesi veya sonrasında immun sistem üzerinde son yıllarda yapılan güncel çalışmalarda ise ruminantlarda özellikle mastitis vakalarında probiyotik uygulamalarının başarılı olduğu görülmüştür. Hayvan yetiştiriciliğinde antibiyotik kullanımının oluşturduğu, başta antibiyotik dirençli bakteri popülasyonlarının artması gibi, önemli sorunlar nedeniyle son probiyotik uygulamalarının özellikle profilaksi amacıyla antibiyotiklere alternatif olarak kullanımı mümkün görülmektedir. Bu derlemede probiyotik mikroorganizmaların hastalıklar bazında etkinliği, immun sistem üzerindeki etkileri güncel çalışmalar ile birlikte ele alınmıştır.

Anahtar kelimeler: Hayvan sağlığı, immun sistem, probiyotik

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Introduction

The term probiotic was first used by Lilly and Stillwell in 1965 as "materials secreted by a microorganism that stimulate the proliferation of another microorganism" and the opposite meaning of the term antibiotic (Kaur et al., 2002). Probiotics were defined by Parker in 1974 as additional complementary foods that have beneficial effects on the intestinal microflora. In 1989, Fuller defined probiotics as "live microbial nutritional supplements that improve intestinal microbial balance for the benefit of the host animal" (Sullivan and Nord, 2002).

The first study on probiotics was made in the late 19th century by the Nobel Prize-winning Russian biologist Elie Metchnikoff, known as the father of probiotics. Metchnikoff established a relationship between fermented milk consumption and longevity and detected the presence of probiotics (lactic acid bacteria) in milk. Metchnikoff observed that Bulgarian villagers who ate yoghurt containing *Lactobacillus* as a regular part of their daily meal consumption had significant longevity and theorized that lactic acid bacteria extended their lifespan (Schrezenmeir and Vrese, 2001).

Microorganisms Used in Probiotics and Selection Criteria

The compound of the gastrointestinal tract flora differs between individuals and within the same individual throughout life. The flora of this system includes both "friendly" and pathogenic bacteria that exist in a complex symbiosis. Many factors such as aging, stress, diet, medication (especially the use of antibacterial), climate, sickness and lifestyle can corrupt this balance, leading to diarrhoea, mucosal inflammation or other serious diseases (Teshale et al., 2017). The pivotal event in the development of a probiotic approach to animal health was the dosing of newly hatched hens with a suspension of gut ingredients designed from healthy adult chickens to detect *Salmonella* spp. in the gut was found to be protected against colonization. Microorganisms used in probiotics include those derived from *Bacillus, Enterococcus, Streptococcus, Clostridium, Lactobacillus, Bifidobacterium species and Escherichia coli* (Kruis et al., 2004). Most probiotic bacteria are lactic acid producing bacteria. Lactic acid has been shown to inhibit coliform growth in the gastrointestinal tract. Acidic flora is harmful to various pathogens. The most widely used probiotic strains include lactic acid bacteria and other Gram-positive bacteria that have been used in food production processes (yogurt, cheese, pickles) for centuries (Henker et al., 2007).

An ideal probiotic should have various potential features that being non-pathogenic and non-toxic by nature, beneficial to the host animal, high viability, stable in storage, capable of surviving or colonizing intestinal tissue, and susceptible for cultivation in an industrial area (Teshale et al., 2017). In addition to these, a probiotic should also have properties stomach acidity, resistance to pancreatic enzymes and bile, ability to adhere to intestinal mucosal cells, high survival rate during transportation during storage, and production of antimicrobial clauses against pathogenic bacteria (Boaventura et al., 2012). In the selection of probiotics, the digestive system of healthy animals or sources of microorganisms such as flowers, rotting fruits and other niches should be selected first. Subsequently, the microorganisms intended to be studied are isolated and identified through selective culture media. A new culture is designed with only target colonies for in vivo evaluation by comparing characteristics such as target species pathogenicity, pathogen inhibition, resistance to host situations. If there are no restrictions on the use of the target species, large and small scale in vivo supplementation experiments are performed to check whether there are real benefits to the host. Finally, probiotics can be produced and used commercially, offering substantial satisfactory results. The main bacteria used in probiotic products were showed in Table 1 (Boaventura et al., 2012). Yeasts are rich in protein, B vitamins, exogenous enzymes and trace elements, and they also have a high degree of digestibility. However, very few yeast species are used commercially. *Saccharomyces cerevisiae*, also known as baker's yeast, is one of the most common commercialized yeast strains (Vanbelle et al., 1990).

Table 1. Bacteria used in probiotic products (Teshale et al., 2017).

Lactobacillus	Bifidobacterium	Other Lactic acid producing bacteria	Non-lactics
L. acidophilus	*B. adolescentis*	*E. faecalis*	*B. cereus*
L. casei	*B. animalis*	*E. faecium*	*E. coli*
L. crispatus	*B. bifidum*	*Sporolactobacillus*	*P. freudenreichii*
L. gallinarum	*B. breve*	*Leuconostoc*	
L. gasser	*B. infantis*	*Mesenteroides*	
L. johnsonii	*B. lactis*	*S. thermophilus*	
L. paracasei	*B. longum*	*P. acidilactici*	
L. plantarum			
L. reuteri			
L. rhamnosus			

Modes of Action of Probiotics

The effect of probiotics was noted only concerning the incidence of the gastrointestinal tract, diarrhea and other intestinal infections (Sullivan and Nord, 2002). However, probiotics general mechanisms of action can be broadly classified as competitive exclusion, bacterial antagonism, and immune modulation (Yirga, 2015).

The competitive exclusion principle is defined as the protective power of normal microflora from the harmful effects of pathogens. This concept is based on the supplementation of the diet of selected cultures of beneficial microorganisms with potentially harmful bacteria for sites of adhesion and organic substrates (main carbon and energy sources). It includes adhesion to the cell wall of the digestive tract, preventing colonization of pathogenic microorganisms or competing for nutrients (Yirga, 2015).

Probiotics show their bactericidal activity effect by fermenting lactose to lactic acid, lowering the pH to a level that harmful bacteria cannot tolerate. In addition to these effects, for example, Lactobacillus species produce hydrogen peroxide and some Enterococcus species prevent the development of pathogenic microorganisms by producing antimicrobial substances such as nisin (McDonald et al., 2010).

Use of Probiotics in Animal Health

Nowadays, probiotic additives are among the chemicals that support growth, especially in animals, and studies that increase resistance against diseases in animals are also supported. Probiotics prevent the deterioration of body balance in animals and increase the development of natural healthy microflora (Boaventura et al., 2012).

Probiotic preparations are in different forms such as powder, granule, pellet, liquid suspension and capsule and can be used by mixing with drinking water or food. Live bacteria, fungi and yeasts used as probiotics have to maintain their viability during storage, application and in the intestinal environment to show their effects (Krehbiel et al., 2003). Probiotic preparations consisting of *Lactobacillus*, *Bifidobacterium* and *Streptococcus* species should be stored at 22-25 °C and in a dry place. They lose their vitality when the storage temperature rises above 30 °C. In addition, yeast *Saccharomyces cerevisiae* and *Bacillus* spp. can withstand the pelleting temperature, while *Lactobacillus*, *Bifidobacterium* and *Streptococcus* spp. depend on the pelleting temperature, significant losses occur. With the microencapsulation method applied to bacteria used as probiotics recently, these bacteria can be made to withstand the pelleting temperature of 90-95° C (Vanbelle et al., 1990). When studies for dogs and cats were examined, it was determined that there were more microorganisms in their gastrointestinal tract than humans. While *Lactobacillus* species have been detected in all intestinal sections in cats and dogs, it has been reported that there are also species found in humans within these *Lactobacillus* species (Grześkowiak et al., 2015).

Pascher et al. (2008) investigated the impacts of *Lactobacillus acidophilus* DSM 13241 in dogs with non-specific dietary sensitivity. As a result of the study, feeding with probiotics improved stool consistency, stool dry matter, and stool frequency. Numerically less *C. perfringens* and *Escherichia* spp. were detected in the stools of dogs given probiotic than those not given (control group). In addition, it was determined that *Lactobacillus* spp. and *Bifidobacterium* spp. were increased numerically in dogs given probiotics. Sauter et al. (2006) examined by adding two lyophilized *Lactobacillus acidophilus* (NCC2628 and NCC2766) and one lyophilized *Lactobacillus johnsonii* (NCC2667) each strain at a density of 10^{10} to their feed for four weeks, in 21 dogs with foodborne diarrhoea. As a result of their study, they found useful impacts on intestinal microbiota and cytokine patterns; the number of enterobacteria in the stool was decreased and the number of *Lactobacillus* spp. increased. Clinical improvement was observed in all dogs treated with probiotics.

Strompfova and Marcinakova (2006) reported that the potential probiotic strain *Lactobacillus fermentum* AD1 isolated from dog feces had high survival (86.54%) at pH 3 in in vitro study and a high adhesion ability to the intestinal layer. *Lactobacillus fermentum* AD1 strain at 10^9/ml was given to the diet of 15 healthy dogs for seven days. As a result of the research, the number of *Lactobacillus* spp. and *Enterococcus* spp. in the stool was significantly increased.

In a study on probiotic efficacy in dogs with inflammatory bowel disease, twenty dogs were treated with probiotic (a mixture of strains belonging to species *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus delbrueckii subspecies bulgaricus*, *Lactobacillus acidophilus*, *Bifidobacterium longum*, *B. breve*, *B. infantis*, and *Streptococcus salivarius subspecies thermophilus*) for 60 days. As a result of these applications, the protective effect of the probiotic significantly reduced CD3 + T cell infiltration as well as positive clinical and histological findings. They also found a normalization of intestinal dysbiosis in dogs treated with probiotics. The result of this study shows that the probiotics used in the treatment of inflammatory bowel disease in dogs can be successful and more research is needed in the field of probiotics and infectious bowel disease (Jergens and Simpsons, 2012). Strompofova et al. (2014) determined that it increased the amount of organic acid in the blood serum of dogs and decreased the amount of triglyceride and albumin in the study they conducted with *Bifidobacterium animalis* B/12 strain (10^9 CFU) of canine origin. In addition, they found an increase in the phagocytic activity of leukocytes. Grześkowiak et al. (2014) found that *Lactobacillus. plantarum* VET14A, *Lactobacillus rhamnosus* VET16A, *Lactobacillus fermentum* VET9A strains isolated from dogs showed successful adhesion to the enteric mucosa. Besides, they stated that it prevents the colonization of widespread enteropathogens such as *Clostridium perfringens*, *Salmonella enterica serovar Typhimurium, Enterococcus canis*, in their in vitro studies.

The probiotic product containing *E. faecium* SF68 increased immune responses to vaccination in puppies and kittens eight to 52 weeks old and seven to 27 weeks old, respectively. Vaccine applications were applied in the first and fourth weeks of the study. An increase in IgA content can be interpreted as a sign of enhanced protection against pathogens. However, increased IgA concentrations may represent a response to antigenic stimulation without increased immunity or to the body's protective mechanism against a noxious stimulus (Veir et al., 2007). *Lactobacillus rhamnosus* GG probiotic strain has many immunomodulatory effects, such as its use as an adjuvant in allergic diseases and vaccines, as well as its effects on gastrointestinal health, especially in newborns (Segers and Lebeer, 2014). In a study conducted to evaluate puppies sensitive to *Dermatophagoides farinae*, those not given probiotics were formed as a control group, and those given *Lactobacillus rhamnosus* GG, which is used to reduce allergic symptoms, were formed as an experimental group. The experimental group was given probiotics containing *Lactobacillus rhamnosus* GG from three weeks to six months. All puppies used in the study were found to be susceptible to *D. farinae.* In the intradermal skin tests of the experimental group using probiotics, lower reaction rates and lower IgE titers were detected in the control group. Since all dogs were sensitive, they were followed and the favourable effect was observed three years after the truncation of the probiotic treatment (Marsella, 2009).

Durand et al. (2006) stated that the number of *Escherichia coli* 0157:H7 was considerably reduced by the application of a probiotic preparation containing *Lactobacillus fermentum*, *Streptococcus faecium*, *L. plantarum* , *L. acidophilus* and *L. casei* in sheep feces.

Lema et al. (2001) researched the influence of *Lactobacillus acidophilus*, *Streptococcus faecium*, a blend of *Streptococcus faecium*, *Lactobacillus acidophilus* and a mixture of *Lactobacillus casei, L. acidophilus*, *S. faecium*, *L. plantarum* and *L. fermentum* in reducing fecal shedding of sheep experimentally infected with *Escherichia coli* O157:H7. As a result of the research, they found that dietary *S. faecium* decreased the fecal shedding of *E. coli* O157:H7. Ohya et al. (2000) investigated the effect of two probiotic bacteria (*Streptococcus bovis* LCB6, *Lactobacillus gallinarum* LCB12) isolated from healthy calves on faecal shedding in calves experimentally infected with *Escherichia coli* O157:H7. As a result of the study, they reported that the treatment of cattle with the probiotics was shown to eliminate fecal shedding of *Escherichia coli* O157:H7 in experimentally infected calves compared to the control group. Mazmanian et al. (2005) reported that polysaccharides produced by *Bacteroides fragilis* in ruminants and some mammals play various immunomodulatory roles in directing maturement of the developing immune system, including correcting systemic T cell deficits, regulating helper T cell 1 and T cell 2 derangements and directing lymphoid tissue biogenesis. A recent study by Donaldson et al. (2018) found that the immunoglobulin A antibody produced by the host in response to the *Bacteroides fragilis* capsule provides a colonization advantage by helping the bacteria bind to the epithelial surface.

The use of probiotics in the dairy industry to treat mastitis of ruminants, especially cattle, is also widely studied and researched. *Lactococcus lactis* DPC 3147 with broad-spectrum antimicrobial properties has been reported to be successful in its activity against pathogens causing mastitis in in vitro studies. When combined with a bismuth-based product, *Lactococcus lactis* DPC 3147 has been found to have a protective effect in cases of mastitis caused by *Staphylococcus aureus* and *Streptococcus dysgalactiae*, which are frequently seen in dried cows (Hu et al., 2019). Klostermann et al. (2008), compared intramammary *Lactococcus lactis* DPC 3147 and antibiotic administration in naturally infected cows with subclinical and clinical mastitis. In antibiotic treatment, prednisolone was used together with amoxicillin-clavulanic acid, which was found to be sensitive to the bacterial agent. Nine out of 25 animals treated with intramammary antibiotics and seven out of 25 animals treated with intramammary live *Lactococcus lactis* DPC 3147 had a similar bacteriological profile at the end of the 12th day. There was no change in the number of somatic cells in the two experimental groups compared to the previous ones. At the end of the research, 15 animals out of 25 treated with *Lactococcus lactis* DPC 3147 and 18 animals out of 25 treated with antibiotics did not show clinical signs of the disease after treatment. According to the research results, they reported that *Lactococcus lactis* DPC 3147 showed that mastitis treatment was possible and that it could be as effective as common antibiotic treatments in some cases.

Armas et al. (2017) investigated in vitro the antagonist activity adhesion and invasion ability of *Lactococcus subsp. lactis* LMG 7930 nisin-producing strain. As a result of the study, they found successful in terms of invasion and adhesion to the cow mammary epithelial cell line. They reported that *Lactococcus subsp. lactis* LMG 7930 as an antagonistic effect inhibited two strains of cow mastitis, *S. aureus* LMG 16805 and *Streptococcus agalactiae* LMG 14838. It failed to inhibit *Escherichia coli* 285-05, *Staphylococcus intermedius* 146-08 and *Streptococcus dysgalactiae* 115-06, *Streptococcus agalactiae* 115-06, *S. aureus* 357-08, *S. epidermidis* 175-07, *S. epidermidis* 200-SA, *S. chromogenes* 100-SA, *Lactococcus cremoris* LMG 7951 strains in sheep.

Pellegrino et al. (2019) 12 probiotic bacteria isolated from milk samples (*Pediococcus pentasacaeous* CRL 1831, *P. pentasacaeous* CRL 1832, *Weissella cibaria* CRL 1833, *W. cibaria* CRL 1840, *Enterococcus hirae* 7-3, *E. hirae* CRL 1834, *E. hirae* CRL 1835, *E. hirae* CRL 1837, *E. mundii* CRL 1656, *Lactococcus lactis* CRL 1655, *L. perolens* CRL 724, *L. plantarum* CRL 1716) and evaluated their antimicrobial effects against selected mastitis agents. *S. aureus* ATCC25923, *S. aureus* RC108, *S. epidermidis* ATCC14990, *S. agalactiae* ATCC27956, *S. dysgalactiae* ATCC27957, *S. uberis* 102, *S. uberis* ATCC27958, *S. hyicus* 112249, *S. bovis* ATCC27960, *Enterococcus faecalis* 1943, *E. faecium*

35667, *Pseudomonas* spp., *Escherichia coli* 345, *E. coli* ATTC35218 *and K. pneumoniae* ATCC10031 were selected as mastitis agents. They rated the antimicrobial effects of bacteria isolated from milk against selected mastitis agents at low, medium and high levels. As a result of the research, it was determined that *L. plantarum* CRL 1716 did not show any antimicrobial activity against mastitis agents except *S. dysgalactiae* ATCC27957, *E. coli* 345, *E. coli* ATTC35218, *Pseudomonas* spp. and *K. pneumoniae* ATCC10031. *Lactococcus lactis* CRL 1655 *E. hirae* CRL 1835, *E. hirae* CRL 1837, *E. mundii* CRL 1656 showed high, other probiotic bacteria showed moderate antimicrobial activity against *S. dysgalactiae* ATCC27957. Antimicrobial activity of all probiotic bacteria isolated from milk against *S. dysgalactiae* ATCC 27957 was the most striking result in this study.

Microorganisms in balance in the gastrointestinal tract of a healthy poultry aid digestion and absorption and increase body resistance against infectious diseases. This balance is disrupted due to stress or illness. In such cases, changes occur in the intestinal flora and thus the balance of the flora is disturbed. The number of lactic acid bacteria in the flora decreases also number of pathogenic bacteria may increase (Koçak et al., 2016). Wang et al. (2017) reported that feeds containing *Bacillus* spp. were more effective in feed conversion rate and body weight gain rate in poultry. They also stated that the intense presence of *Firmicutes* species increased the accumulation of acetate in the cecum and the application of *Lactobacillus casei* in broiler chickens was beneficial for the health and development of chickens by reducing the urease activity in the ileum.

Torshizi et al. (2010) stated that the incidence of Salmonellosis in broilers decreased significantly when a commercial product containing *Lactobacillus casei*, *L. acidophilus*, *Enterococcus faecium* and *Bifidobacterium bifidium* was given together with feed or drinking water. In the same study, they found that there was a significant improvement in the experimental groups compared to the control groups in terms of body weight and feed conversion rates on the 31st day. Kergourlay et al. (2012) reported the draft genome sequence of *Lactobacillus salivarius* SMXD51 isolated from the cecum of healthy chickens, showing activity against *Campylobacter jejuni*, the most common cause of Campylobacteriosis infection. As a result of the study, they determined that *Lactobacillus salivarius* SMXD51 has interesting properties as a potential probiotic strain. Santini et al. (2010) investigated the antimicrobial activity of 55 isolates (lactic acid producing bacteria and Bifidobacterium species) against *Campylobacter jejuni* LMG 8842, *C. jejuni* CIP70.2, and *C. jejuni* 221/05 strains. As a result of the study, they determined that *Bifidobacterium longum subspecies longum* PCB 148, *B. longum subsp. longum* PCB 133, *B. breve* PCB 110, *B. pseudocatenulatum* PCB 107, *B. longum subsp. infantis* PCD 889B, *B. thermophilum* PCD 359B, *B. longum subsp. longum* PCD 232B, *L. plantarum* PCS 20, *Lactobacillus* spp. PCK 161, *L. pseudomesenteroides* PCK 18, *L. plantarum* PCA 306, *L. plantarum* PCA 293, *L. plantarum* PCA 275, *L. plantarum* PCA 259, *L. plantarum* PCA 236, *L. pentosus* PCA 227 showed antimicrobial activity against three of the *C. jejuni* LMG 8842 , *C. jejuni* CIP70.2 and *C. jejuni* 221/05 strains. Elraheam Elsayed et al. (2021) investigated the beneficial effects of a potential synbiotic (*Lactobacillus delbrueckii subspecies bulgaricus*, *L. plantarum, L. acidophilus*, *L. rhamnosus*, *B. bifidum*, *E. faecium*, *S. thermophilus*, *Aspergillus oryzae*, *Candida pintolepesii*) with a concentration of 2 × 10^9 cfu/g, commercial product (%90 lactic acid, %10 formic acid) and multi-strain bacterin formulated from avian pathogenic multidrug-resistant *Escherichia coli* O26, O78, *S. Enteritidis* and *S. Typhimurium* serotypes. They performed a challenge test against *E. coli* O26, O78, *S. Enteritidis* and *S. Typhimurium* after eight days of using that three products together or alone. As a result of the study it was determined the combined use of that three products, especially when applied on the first day, mortality, developed erythrogram parameters, produced the immunomodulatory effect, decreased proinflammatory cytokine levels and enhanced growth performance parameters. Talebi et al. (2014) investigated the antibody response of the probiotic commercial product (*L. acidophilus, L. casei, E. faecium, B. bifidium*) to the Newcastle and Gumboro vaccine. In both vaccine administrations, maternal antibodies decreased to normal levels by 21 days, but the decrease in titer was slower in the probiotic-treated groups than in the vaccinated or control groups. Stefaniak et al. (2020) investigated the early in ovo administration (on day 12 of embryo incubation) of selected synbiotics in broilers that affects the humoral immune response to experimental antigens. They found that the in ovo application of synbiotic (inulin and *Lactococcus lactis susbsp. Lactis*) did not significantly influence the humoral immune response against T cell-dependent antigen and IgG value. However at the end of the 35 days, they reported that the while mortality rate was 8.5% in the control group, it was 2.1% in the synbiotic-administered experimental group. Wu et al. (2019) investigated the impacts of *Enterococcus faecium* NCIMB 11181 on the growth performance and immune reaction of broilers. They found an increase in growth and antibody response at the rates of 1 × 10^8 and 2 × 10^8 CFU/kg added to the daily feed of the broilers, but they determined the highest amount of IgG in the serum on the 35th day in the other experimental group at the rate of 5 x 10^7 CFU/kg. Koenen et al. (2004) reported that chickens fed diets containing liquid Lactobacillus induced higher IgG and IgM responses compared to the others. In a different study, Huang et al. (2004) stated that when they applied *Lactobacillus acidophilus* and *Lactobacillus casei* in the feed they consumed daily, they detected a higher IgA response compared to the control group, while the IgG value was not affected.

Conclusion

Probiotic applications are at the forefront of the methods applied in recent years due to important problems

caused by the use of antibiotics in animal husbandry, such as the increase in populations of antibiotic resistant bacteria. Although probiotics are not expected to replace antibiotics in the treatment of an acute disease, it seems possible to use them as an alternative to antibiotics for prophylaxis and growth performance in animals. In recent years, studies on probiotics in ruminants have been conducted in mastitis cases and have shown that intramammary applications can be as effective as antibiotics.

The impacts of probiotics, prebiotics and synbiotics on the immune system and its components have been seen positive in some studies on cats and dogs and poultry, and it has been concluded that longer-term evaluation is required in scientific studies to see their effects in other species.

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Conflict of interest

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

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