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RESEARCH ARTICLES

CASE REPORTS

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RESEARCH ARTICLE

The Effects of Prepartum Vitamin E, Selenium and Melatonin Treatment on Uterine Involution of Awassi Sheep in Hatay

Ahmet GÖZER[1*], Onur BAHAN[2], Gökhan UYANIK[3], Ufuk KAYA[4], Ebru ARSLANHAN[1], Büşra KÜÇÜKKARA[1], Gönül TATAR[1], Ramazan SERTKOL[1], Mustafa Kemal SARIBAY[1], Gökhan DOĞRUER[1]

[1]Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, Hatay, Türkiye
[2]Yozgat Bozok University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, Yozgat, Türkiye
[3]Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Internal Medicine, Hatay, Türkiye
[4]Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Biostatistic, Hatay, Türkiye

ABSTRACT

The aim of this study was to investigate the effects of prepartum Vitamin E, Selenium and Melatonin treatments on uterine involution in Awassi sheep. The study included a total of 24 ewes with singleton pregnancies. Fifteen days before their expected delivery, ewes were randomly divided into three groups. Group VE (n=8) received Vitamin E and Selenium intramuscularly. Group M (n=8) received a subcutaneous ear implant containing 18 mg of melatonin. Group C (n=8) served as the control group and received no treatment. The mean time for completion of uterine involution in Group VE, Group M, and Group C were 20.57±1.21; 19.13±1.13; 18.67±1.09 days, respectively ($p>0.05$). Uterine diameters were 9.66±0.59; 9.54±0.35; 7,95±0.22 in Group VE, Group M, and Group C, respectively. Uterine diameters in Group VE and Group M were significantly greater than Group C on Day 3 of postpartum ($p<0.05$). Mean outer caruncular diameters were 1.93±0.10; 1.60±0.10; 1.65±0.07 in Group VE, Group M, and Group C. respectively. Mean outer caruncular in Group VE were significantly greater than Group M and Group C ($p<0.05$). Mean outer caruncular diameters were greater on Day 3 compared to Day 6 regardless of groups ($p<0.05$). Mean inner caruncular diameters were greater on Day 3 compared to Day 6 regardless of groups ($p<0.05$). There was no differences in terms of mean inner caruncular diameters between groups ($p>0.05$). In conclusion, it was found that prepartum administration of Vitamin E, Selenium and Melatonin had no significant effects on the completion time of postpartum uterine involution on Awassi ewes.

Keywords: Melatonin, Selenium, Sheep, Uterine Involution, Vitamin E

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İvesi Irkı Koyunlarda Prepartum Vitamin E, Selenyum ve Melatonin Tedavisinin Uterus İnvolüsyonu Üzerine Olan Etkileri

ÖZ

Bu çalışmanın amacı, prepartum dönemde Vitamin E, Selenyum ve Melatonin uygulamalarının İvesi ırkı koyunlarında uterus involüsyonu üzerine etkilerini araştırmaktı. Çalışmaya tekil gebeliğe sahip toplam 24 koyun dahil edildi. Beklenen doğum zamanlarından 15 gün önce koyunlar rastgele üç gruba ayrıldı. Grup VE'deki koyunlara (n=8) intramüsküler yoldan Vitamin E ve selenyum uygulandı. Grup M'deki koyunlara (n=8) 18 mg melatonin içeren subkutan kulak implantı uygulandı. Grup C'deki koyunlar (n=8) ise kontrol grubu olarak kullanıldı ve bu gruba herhangi bir tedavi uygulanmadı. Uterus involüsyonlarının tamamlanma süresi Grup VE, Grup M ve Grup C için sırasıyla 20,57±1,21; 19,13±1,13; 18,67±1,09 gün olarak tespit edildi ($p>0,05$). Uterus çapları Grup VE, Grup M ve Grup C için sırasıyla 9,66±0,59; 9,54±0,35; 7,95±0,22 olarak belirlendi. Doğum sonrası 3. günde Grup VE ve Grup M'deki uterus çapları, Grup C'den anlamlı derecede büyük tespit edildi. ($p<0,05$). Ortalama dış karunkular çapları Grup VE, Grup M ve Grup C için sırasıyla 1,93±0,10; 1,60±0,10; 1,65±0,07 olarak ölçüldü. Grup VE'deki ortalama dış karunkular çapları, Grup M ve Grup C'den anlamlı derecede büyük olduğu görüldü ($p<0,05$). Gruplardan bağımsız olarak; 3. gündeki ortalama dış karunkular çapları, 6. güne kıyasla daha büyük olarak tespit edildi ($p<0,05$). Aynı şekilde 3. gündeki iç karunkular çapları da 6. güne kıyasla daha büyük olarak tespit edildi ($p<0,05$). Gruplar arasında ortalama iç karunkular çapları açısından fark tespit edilmedi ($p>0,05$). Sonuç olarak, prepartum dönemde Vitamin E, Selenyum ve Melatonin uygulamasının, İvesi koyunlarında postpartum uterus involüsyon süresinin tamamlanması üzerinde önemli bir etkisinin olmadığı görüldü.

Anahtar Kelimeler: Koyun, Melatonin, Selenyum, Uterus İnvolüsyonu, Vitamin E

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ORCID ID; *AG: 0000-0001-8658-5916, OB: 0000-0003-0878-6338, GU: 0000-0003-4488-3055, UK: 0000-0002-4805-0993, EA: 0000-0002-6417-9041, BK: 0000-0002-0686-6097, GT: 0000-0001-8374-3499, RS: 0000-0003-2456-8146, MKS: 0000-0002-9903-4942, GD: 0000-0002-8000-0646*

*Corresponding author e-mail: ahmetgozer@mku.edu.tr

INTRODUCTION

In sheep, the postpartum period commences immediately following the expulsion of the placenta after parturition and continues until the genital organs return to their pre-gravid state. This period encompasses key physiological processes, including endometrial restoration, elimination of bacterial contamination, resumption of ovarian cyclicity, and uterine involution (Noakes 2001). Uterine involution is generally completed between 17 and 21 days postpartum (Rubianes and Ungerfeld 1993; Fernandes et al. 2013), though it may extend to 28–35 days postpartum depending on factors such as parity and the occurrence of dystocia (Hauser and Bostedt 2002; Zdunczyk et al. 2004; Fernandes et al. 2013). In sheep, the uterine involution is also influenced by a multitude of factors, including suckling intensity, season of lambing, delivery type and frequency, nutritional status, milk production levels, litter size, and postpartum body weight loss (Mbyahaga et al. 1998; Hayder and Ali 2008; Hauser and Bostedt 2002; Pollott and Gootwine 2004; Medan and El-Daek 2015; Liu et al. 2022).

In traditional sheep production systems, monitoring postpartum uterine involution and the initiation of ovarian cyclicity is rarely prioritised due to characteristics of the reproductive physiology in ewes. However, with the increasing adoption of extensive production systems, the acceleration of uterine involution and stimulation of ovarian activity in the postpartum period have gained importance. Shortening the postpartum anestrus period and accelerating the postpartum uterine involution is one of the viable strategies for enhancing lambing rates and overall reproductive efficiency (Morris 2017). Therefore, several attempts have been made to accelerate uterine involution and induce ovarian activity in the postpartum period in ewes. Gonadotropin-releasing hormone (GnRH) and nutritional supplementation during the postpartum period effectively stimulate ovarian function (Mitchell et al. 2003). Additionally, specific hormonal treatments, including prostaglandin F2α (PGF2α) (Dal et al. 2020), carazolol (Enginler et al. 2023), and oxytocin (Li et al. 2025) have been shown to expedite uterine involution when administered during the postpartum period. Also, the effects of Melatonin, Vitamin E and Selenium on various post-partum reproductive performance parameters, including uterine involution, in goats and sheep have been studied in many studies (Abdel-Raheem et al. 2019; Afzaal et al. 2023).

Melatonin, a hormone secreted by the pineal gland, plays a pivotal role in regulating various reproductive processes, including the hypothalamic-pituitary-gonadal axis, placental function, fetal development, ovarian follicular dynamics, corpus luteum activity, ovulation, and embryo development (Reiter et al., 2009). The effects of melatonin on the reproductive axis are seasonal, primarily promoting the release of luteinizing hormone-releasing hormone (LHRH). Subcutaneous administration of melatonin has been shown to increase the frequency of LHRH release and luteinizing hormone (LH) pulses, achieving approximately 10 pulses per 6 hours after a delay of 40–50 days (Malpaux et al. 1996). In small ruminants, melatonin treatment is widely employed for estrus synchronization, enhancing twin survival rates (Flinn et al. 2020), and regulating ovarian activity (Kusakari and Ohara 1997; Afzaal et al. 2023). For instance, in a study conducted on Payoya goats, subcutaneous melatonin administration significantly increased estrus response, ovulation rates, fertility, and conception rates (Zarazaga et al. 2013). Similarly, melatonin supplementation in postpartum Suffolk sheep reduced the interval to estrus and ovulation (Kusakari and Ohara 1997). Furthermore, melatonin administration during the prepartum period has been reported to enhance postpartum reproductive performance by shortening the time to first postpartum ovulation and expediting placental expulsion (Afzaal et al. 2023).

Vitamin E and Selenium are important units of antioxidant system, which prevents peroxide radical formation within cell membranes by neutralizing peroxides and hydroperoxides. Vitamin E plays pivotal roles in oocyte maturation, oocyte quality, fertilization, and early embryonic development (Kott et al., 1998). Deficiency of Vitamin E results in several reproductive disorders, such as early embryonic death, fetal resorption, stillbirths, and fetal muscular dystrophy (Kott et al. 1998; Kaçar et al. 2008). Selenium, a vital component of the endogenous antioxidant defense system, exerts its effects through its role in the activity of glutathione peroxidase (GSH-Px), an enzyme essential for mitigating oxidative stress (Köse et al. 2013). Selenium deficiency is related with infertility, anestrous, retained placenta, abortion and stillbirth in dairy cows (Kamada 2016; Uematsu et al. 2016). Selenium supplementation increased progesterone concentrations and decreased incidence of metritis, ovarian cyst, and retained placenta (Spears and Weiss 2008; Wei et al. 2022). In ewes, Vitamin E and selenium supplementation improves the estrus, fertility, and prolificacy rates (Efe et al. 2023; Samimi et al. 2023; Semra et al. 2023). Vitamin E and selenium supplementation during late pregnancy is also associated with improvement in the lamb survivals (Zanghishe et al. 2023). Similarly, in goats, Vitamin E and selenium supplementation has been reported to improve uterine health, suggesting its potential as a novel tool for enhancing fertility and pregnancy outcomes (Alalaf and Alnuaimy 2024).

Although the effects of Vitamin E, selenium, and melatonin on pregnancy rates, lamb survival, and certain reproductive parameters are well-documented, research on their impact on uterine involution remains limited. This study examines the effects of prepartum supplementation with Vitamin E, selenium, and

melatonin on postpartum uterine involution in Awassi sheep in Hatay

MATERIALS and METHODS

Ethics Approval and Location of The Study

This study was approved by the Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee for Animal Experiments with the decision numbered 2024/01-06 on 26/01/2024. The present study was conducted on a sheep farm located in Reyhanlı District of Hatay province of Türkiye (latitude 36°25' N and longitude 36°53'E) between June 2024 and January 2025.

Estrus Synchronization, Experimental Design and Vitamin E, Selenium and Melatonin Treatment

Vaginal sponges containing 60 mg medroxyprogesterone acetate (Esponjavet®, HIPRA, Spain) were inserted into the vagina of 50 sheep in their reproductive season, aged 1 year and weighing 45-60 kg, using a special speculum for estrus synchronization. The sponges were kept in the vagina for 12 days and 500 IU PMSG (Chronogest/PMSG, 6000 IU, Intervet, Istanbul, Turkiye) and 0.075 mg d-cloprostenol (Senkrodin®, Vetaş, Turkiye) were administered intramuscularly at sponge withdrawal. The rams were introduced to the flock 24 hours after the sponge removal, with sessions lasting one hour each, twice a day, in the morning and evening. The ear tag numbers of sheep detected in estrus and successfully mated were recorded, and these sheep were separated into different pen. Pregnancy examinations were performed 50 days post-mating using a real-time ultrasound device (Falco, Pie Medical, Netherlands) with a 5-8 MHz transabdominal probe. Twenty-four pregnant sheep with single fetus were randomly selected. These selected sheep were divided into three groups using a random sampling method 15 days before their expected delivery dates. Group VE (Vitamin E and Selenium) (n=8) received 2 ml of selenium and vitamin E (1 mg/ml sodium selenite and 60 mg/ml vitamin E, Yelvit®, Teknovet, Türkiye) intramuscularly. Group M (Melatonin) (n=8) received an ear implant containing melatonin (18 mg melatonin, Regulin®, Ceva, Türkiye). Group C (Control) (n=8) received no treatment and left as a control group. All deliveries occurred in November and December among the 24 pregnant Awassi sheep. The number of offspring born was recorded, and only sheep giving birth to a single lamb were enrolled in the study. Estrus synchronization protocol, treatments and groups were illustrated in Figure 1.

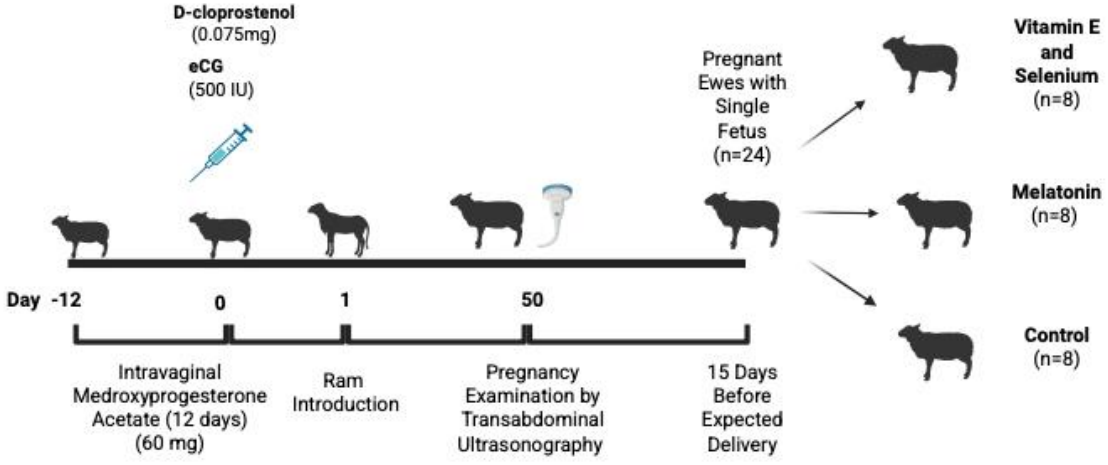

Figure 1. Schematic illustration of the estrus synchronization protocol, treatments and groups in ewes

Ultrasonographic Examination

Starting from the third day postpartum, uterine involution was monitored during the first three weeks postpartum at 3-day intervals using a real-time ultrasound device with a convex 5–8 MHz probe (Falco, Pie Medical, Netherlands). During the first 10 days postpartum, transabdominal ultrasonography was performed using a 5 MHz frequency. After the 10th day postpartum, transrectal ultrasonography was conducted with linear probe (5-8 MHz frequency). For transabdominal ultrasonography, the procedure was carried out while the sheep were standing. Before the examination, ultrasound gel was applied to the examination site. In the transrectal ultrasonography method, feces were removed from the rectum using a lubricated sterile glove before inserting the lubricated ultrasound probe into the rectum. During both transabdominal and transrectal examinations, the maximum diameter of the uterine horns was measured. For the measurement of caruncular diameters, three caruncles were evaluated, and their internal and external dimensions were recorded (Figure 2a). Uterine involution was considered complete when the transverse diameter of the uterus was less than 2 cm and the uterine lumen was empty (Figure 2b). The postpartum uterine ultrasonographic examinations were illustrated in Figure 3.

Statistical Analysis

Descriptive statistics for each variable were calculated and presented as mean ± std. error (SEM). Kaplan Meier survival analysis was used to calculate mean uterine involution time and life curves. To test the difference between the life curves, the Breslow test was used. A linear mixed model for repeated measures was used to test the differences in each caruncle. was used to test the differences in each caruncle. Animals were included as a random factor in all models while group, sampling time and their interaction were included as a fixed factor. Pairwise comparisons were done using a Bonferroni adjustment. $p<0.05$ was considered significant in all analyses. All statistical analyses were performed by using the IBM SPSS 23.0 package programme for Windows.

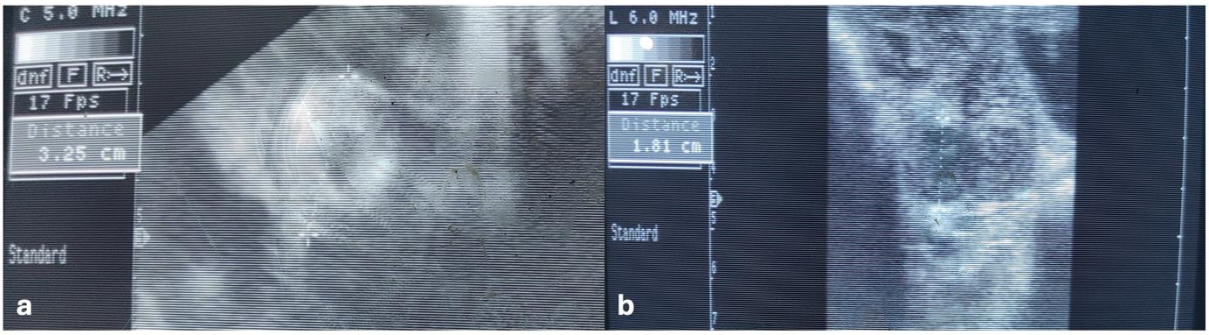

Figure 2. a. outer caruncle diameter b. cornu uterine diameter

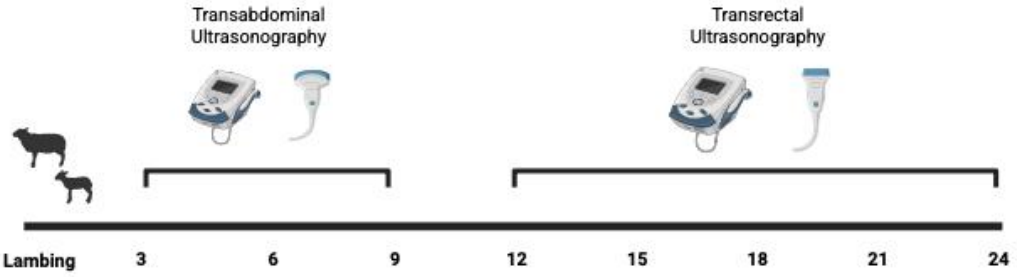

Figure 3. Schematic illustration of the postpartum uterine ultrasonographic examination in ewes

RESULTS

Uterine Horn Diameter Difference

In Group VE, a statistically significant reduction in the uterine horn diameters was observed during the first 3–12 days postpartum (pp) ($p<0.05$). However, after the 12th day postpartum, although a decrease in the uterine horn diameters was noted, no statistical difference was detected ($p>0.05$) (Table 1). The time required for the completion of uterine involution in Group VE was determined to be 20.57±1.21 days (Table 2). In Group M, a statistically significant reduction in the uterine horn diameters was observed during the first 9 days postpartum ($p<0.05$). However, after the 9th day postpartum, although a reduction was observed, no statistical difference was detected ($p>0.05$) (Table 1). The time required for the completion of uterine involution in Group M was determined to be 19.13±1.13 days (Table 2). In Group C, although the reduction in the diameter was observed, no statistically significant decrease in the uterine horn diameters was observed during the first 6 days postpartum ($p>0.05$). However, after the 6th day postpartum, a statistically significant reduction was detected ($p<0.05$) (Table 1). The time required for the completion of uterine involution in Group C was determined to be 18.67±1.09 days.

Caruncular Diameter Difference

The outer caruncular diameters were 2.10±0.12; 1.85±0.12; 1.67±0.09 cm and 1.76±0.13; 1.34±0.11; 1.63±0.12 in Group VE, Group M, and Group C, respectively on Day 3 and Day 6 ($p>0.05$). The mean diameters of the outer caruncle were 1.86±0.07 and 1.57±0.08 respectively on Day 3 and Day 6 ($p<0.05$). The inner caruncular diameters were 0.87±0.07; 0.73±0.08; 0.69±0.06 cm and 0.64±0.05; 0.53±0.04; 0.58±0.06 in Group VE, Group M, and Group C, respectively on Day 3 and Day 6 ($p>0.05$). The mean diameters of the inner caruncle were 0.75±0.04 and 0.58±0.03 respectively on Day 3 and Day 6 ($p<0.05$) (Table 3).

Table 1. The mean diameter of the cornu uteri in Group VE, Group M and Group C within 18 days of postpartum.

Parameter	Group	Time						p		
		Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Group	Time	G*T
Uterine Diameter	Group VE	9.66±0.59 a, A	7.27±0.51 b	4.24±0.34 c	2.60±0.17 d	2.34±0.15 d	2.19±0.17 d	0.020	<0.001	0.017
	Group M	9.54±0.35 a, A	6.69±0.37 b	3.46±0.42 c	2.57±0.18 cd	2.29±0.15 cd	1,.94±0.12 d			
	Group C	7.95±0.22 a, B	7.41±0.41 a	3.35±0.30 b	2.47±0.09 bc	2.13±0.08 c	2.03±0.08 c			

A,B; a-d: Different superscripts indicates the statistical significance among the groups ($p<0.05$)

Table 2. Means for survival time of uterine involution

Groups	Mean[a]			
	Day	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Group VE	20.57	1.21	18.20	22.95
Group M	19.13	1.13	16.92	21.33
Group C	18.67	1.09	16.53	20.81

Kaplan-Meier, Breslow Test; p= 0.504

Table 3. Mean caruncular diameter in the postpartum period

Parameter	Group	Time		LS Mean (Group)	p		
		Day 3	Day 6		Group	Time	G*T
Outer caruncular	VE	2.10±0.12	1.76±0.13	1.93±0.10 A	0.019	0.003	0.108
	M	1.85±0.12	1.34±0.11	1.60±0.10 B			
	C	1.67±0.09	1.63±0.12	1.65±0.07 AB			
LS Mean (Time)		1.86±0.07 a	1.57±0.08 b				
Inner caruncular	VE	0.87±0.07	0.64±0.05	0.75±0.05	0.109	0.001	0.616
	M	0.73±0.08	0.53±0.04	0.63±0.05			
	C	0.69±0.06	0.58±0.06	0.63±0.04			
LS Mean (Time)		0.75±0.04 a	0.58±0.03 b				

A,B; a-d: Different superscripts indicates the statistical significance among the groups ($p<0.05$)

DISCUSSION

There are several reports indicating that uterine involution in the ewes completed within the 17-28 days of postpartum (Rubianes and Ungerfeld 1993; Hauser and Bostedt 2002, Fernandes et al. 2013). However, uterine involution can extend to by the 35th day postpartum in case of dystocia or assisted delivery (Hauser and Bostedt 2002). Hauser and Bostedt (2002) also observed that 80% of the uterus regresses by the 11th day postpartum, while Ahmet et al. (2016) reported that in Awassi sheep, 50% of uterine regression occurs between the 3rd and 14th postpartum days. In the present study, uterine involution completed within the first 23 days of postpartum regardless of the homonal treatments. Prepartum Vitamin E, Selenium and melatonin treatments have no effects on the completion time of uterine involution in Awassi ewes. Decrease in the postpartum uterine diameter in the first 12 days of postpartum was significant in Group VE, Group M, and Group C whereas changes in the uterine diameters beyond the 12th day postpartum was stable, which is compatible with the previous studies (Hauser and Bostedt 2002; Ahmet et al. 2016).

Prepartum Vitamin E and Selenium treatments are widely implemented in large animals to counteract oxidative stress-related diseases, such as mastitis, ovarian cysts, retained placenta, and postpartum metritis in the periparturient period (Xiao et al. 2021). In the prepartum period, Vitamin E and selenium treatments are also carried out in sheep (Milewski et al. 2021; Zanghishe et al. 2023) and goats (Barcelos et al. 2022; Nurmala et al. 2024) to improve lamb survival, colostrum quality and immune system of the ewes. In addition, Vitamin E and Selenium treatments are also used for enhancing reproductive performances. Abdel-Raheem et al. (2019) stated in Ossimi sheep that prepartum Vitamin E and Selenium treatment results in faster uterine involution but does not affect the duration of the completion of the uterine involution. In the same study, treatments caused the earlier resumption of ovarian function and the ovulation of large-size ovulatory follicles and the increase in the small, medium, and large-sized follicles. In the present study, complete uterine involution in ewes is not affected by the Vitamin E and Selenium treatment, as previously described by Abdel-Raheem et al. (2019).

In the present study, the uterine diameter was greater in Group VE and Group M compared to Group C on day 3 postpartum. However, on Day 6 postpartum, there was no significant differences between groups in terms of uterine diameter. Results of the present study indicated that hormonal treatment of melatonin, Vitamin E and selenium lower the uterine involution on the first 3 postpartum days, whereas uterine diameters were similar on day 6 of postpartum. The greater uterine diameter observed in the melatonin group may be related with effect of melatonin on prostaglandin synthesis (Abecia et al. 1999), steroid hormone receptors (Vazquez et al. 2013), and endometrial hemodynamics (Abdelnaby et al. 2020). Vázquez et al. (2013) reported that melatonin implants increase the expression of progesterone receptors (PR) in the deep glandular epithelium during the postpartum period in sheep, thereby enhancing sensitivity to progesterone. In contrast, they found that melatonin reduces the expression of estrogen receptors (ER) in the deep stroma, thereby weakening the luteolytic effect of the estrogen-ER complex. They suggest that this may contribute positively to the preparation of the uterus for pregnancy. Association of melatonin with inhibition of prostaglandin, which is a uterotonic agent, synthesis is well known effect of melatonin (Abecia et al. 1999; Fierro et al. 2013). Melatonin also causes an increase in blood supply to ovarian and uterine arteria (Abdelnaby et al. 2020) and inhibits the apoptosis in the sheep endometrial epithelial cells by regulating the estrogen function (Duan et al. 2023). Melatonin also improves the angiogenesis in caruncular endometrium in early pregnancy in ewes (Viola et al. 2024). All these abovementioned factors may be related with the greater uterine diameter in the Group ME compared to Group C.

In the present study, the uterine diameter was greater in Group VE compared to Group C on day 3 postpartum. However, on Day 6 postpartum, there was no significant differences between groups in terms of uterine diameter. Also, the outer caruncle diameter was greater in Group VE compared to Group C, which is expected and compatible with the literature (Hauser and Bostedt 2002). Caruncule, cotyledon, and placental weights are affected by several factors, such as nutritional restriction and nutritional supplementation. Several reports indicate the conflicting results of the effect of the Vitamin E and Selenium supplementations. Selenium deficiency caused a decrease in the placental size of sheep (Freer and Dove 2002) whereas prepartum adequate and high levels of selenium supplement did not change placentom number, mass, and caruncular and cotyledonary weight (Lekatz et al. 2010). Vural et al. (2008) also stated in ewes that Vitamin E treatments have no effects on the caruncle count and measurements. Therefore, greater diameters that were observed in the Vitamin E and Selenium group were unexpected. In a study conducted on the women concluded that Vitamin E supplement caused improvement in the uterine vascularity and increase in the endometrial thickness (Tasasaki et al. 2010). In a histological study carried out in goat by Alalaf and Alnuaimy (2024), it was observed that Vitamin E and selenium treatments caused larger caruncles and thicker myometrial musculature by improving the epithelial disintegration, uterine glands integrity, and uterine microvasculature. So, greater outer caruncle diameters might be related with effect of Vitamin E and Selenium on the uterine's epithelial, glandular and vascular system. Further research is needed to elucidate the interaction between Vitamin E, Selenium and uterine physiology.

CONCLUSION

In conclusion, prepartum Vitamin E, Selenium and Melatonin treatment did not cause any statistical differences on the uterine involution completion time. In all groups, uterine involution time was found to be completed within literature's values. Although caruncular diameters were also not affected by the treatments, mean caruncular diameter were greater in ewes treated with selenium, which may be caused by Vitamin E and Selenium effect's of uterine physiology. Further studies are needed to demonstrate effect of Vitamin E, selenium and melatonin on postpartum physiology in ewes.

Author's Contributions: AG, OB, GU, and EA contributed to the project idea, design and execution of the study. AG, OB, GU, EA, RS, BK, GT, MKS, GD contributed to the acquisition of data. AG and UK analysed the data. AG, OB and GU drafted and wrote the manuscript. MKS, GD, OB and GU reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Ethical Approval: This study was approved by the Hatay Mustafa Kemal University Local Ethics Committee for Animal Experiments with the decision numbered 2024/01-06 on 26/01/2024.

Conflict of Interest: The authors have no conflicts of interest to report

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RESEARCH ARTICLE

Clinical, Biochemical, Radiographic and Thermographic Evaluation of Extremity Fractures in Calves

Yusuf KOÇ[1*], Zülfükar Kadir SARITAŞ[1]

[1]*Department of Surgery, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Türkiye*

ABSTRACT

This study aim to assess extremity fractures in calves aged 0-6 months by clinical, thermographic, and radiographic methods. A total of 26 patients were assessed clinically, thermographically, and radiographically. Thermographic assessments were conducted using a thermography apparatus. Radiographic evaluations of fracture cases were conducted, and the fractures were categorized. Serum calcium levels in the control and fracture groups were 10.60±0.25 and 11.67±0.23 mg.dl-1, respectively, with the increase in fractures being statistically significant ($p<0.05$). The TNF-α measurement levels were recorded as 0.11±0.01 and 0.15±0.05 pg.ml-1 in the respective groups, with the increase in fractures being statistically significant ($p<0.05$). The IL-1β measurement levels were recorded as 18.67±4.71 and 30.69±7.53 pg.ml-1, respectively, with the increase in fractures being statistically significant ($p<0.05$). The IL-6 measurement levels were recorded as 61.79±5.52 and 98.29±31.85 pg.ml-1, respectively, with the increase in fractures being statistically significant ($p<0.05$). Cortisol measurement values were established at 3.36±0.54 and 4.93±0.97 mcg.dl-1, with a statistically significant increase in fracture cases ($p<0.05$). A thermographic assessment of fracture cases revealed an elevation of 4.14±2.2 °C along the fracture line. Fractures resulting from dystocia and trauma in calves are significant among calf surgical conditions. It was determined that thermography may serve as a diagnostic tool in fracture cases, and further comprehensive investigations are required for its application in the postoperative period.

Keywords: Calf, Fracture, Radiography, Thermography

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Buzağılarda Ekstremite Kırıklarının Klinik, Biyokimyasal, Radyografik ve Termografik Olarak Değerlendirilmesi

ÖZ

Bu çalışmada 0-6 aylık yaş aralığındaki buzağılarda ekstremite kırıklarının klinik, biyokimyasal, termografik ve radyografik olarak değerlendirilmesi amaçlanmıştır. Toplam 26 olgu klinik, termografik ve radyografik olarak değerlendirilmiştir. Termografi cihazı ile termografik incelemeler gerçekleştirilmiş ve kırık olgularının radyografik incelemeleri yapılarak kırıklar sınıflandırılmıştır. Serum kalsiyum ölçüm değerleri kontrol ve kırık gruplarında sırasıyla; 10.60±0.25, 11.67±0.23 mg.dl-1 olarak belirlenmiş ve kırık olgularındaki artış istatistiksel olarak anlamlı bulunmuştur ($p<0.05$). TNF-α ölçüm değerleri gruplarda sırasıyla 0.11±0.01, 0.15±0.05 pg/ml olarak belirlenmiş ve kırık olgularındaki artış istatistiksel olarak anlamlı bulunmuştur ($p<0.05$). IL-1β ölçüm değerleri sırasıyla 18,67±4,71, 30,69±7,53 pg.ml-1 olarak belirlenmiş ve kırık olgularındaki artış istatistiksel olarak anlamlı bulunmuştur ($p<0,05$). IL-6 ölçüm değerleri sırasıyla 61,79±5,52, 98,29±31,85 pg.ml-1 olarak belirlenmiş ve kırık olgularındaki artış istatistiksel olarak anlamlı bulunmuştur ($p<0,05$). Kortizol ölçüm değerleri sırasıyla; 3,36±0,54, 4,93±0,97 mcg.dl-1 olarak belirlenmiş ve kırık olgularındaki artış istatistiksel olarak anlamlı bulunmuştur ($p<0,05$). Kırık olgularının termografik incelemesinde kırık hattında 4,14±2,2 ℃'lik istatistiksel olarak anlamlı artış kaydedilmiştir. Buzağılarda distosi ve travmaya bağlı gelişen kırıklar, buzağı cerrahi hastalıkları arasında önemli bir yere sahiptir. Termografinin kırık olgularında da tanı yöntemi olarak kullanılabileceği ve termografik muayenenin cerrahi sağaltımı izleyen dönemde kullanımına ilişkin daha ayrıntılı çalışmalara ihtiyaç olduğu sonucuna varılmıştır.

Anahtar kelimeler: Buzağı, Kırık, Radyografi, Termografi.

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ORCID ID; *YK: 0000-0002-6342-5466 ZKS: 0000-0002-7659-6635*

*Corresponding author e-mail: yusufkoc@aku.edu.tr

INTRODUCTION

Improper manipulation or the application of manual and mechanical forces by owners and occasionally veterinarians during delivery can result in traumatic injuries to muscles, bones, joints, nerves, and other soft tissues, frequently leading to fetal demise during delivery or the postpartum period (Aksoy et al., 2009). Fractures of the extremities are commonly observed in calves (Aksoy et al., 2009; Belge et al., 2016). Fractures in calves commonly occur in the femur (14%), metacarpus (50%), metatarsus (50%) (Bilgili et al., 2008; Nichols et al., 2010; Rodrigues et al., 2012), radius-ulna (7%), and infrequently in the vertebra (7%). Fractures of the metacarpus occur with double the frequency of metatarsal fractures (Arıcan et al., 2014). X-ray offers essential insights for diagnosis, treatment, and prognosis of vital information. It induces several positions, particularly from lateromedial and caudo-cranial orientations (Ewoldt et al, 2003). Fractures of the metacarpals in calves predominantly occur at the distal epiphysis and metaphysis. Salter-Harris Type I fractures frequently occur in the distal epiphysis and metaphysis of cattle. The cortex considerably narrows at the transition from diaphysis to metaphysis, resulting in restricted axial strength in this region of the metacarpus (Belge et al, 2016). The management of fractures in calves holds significant relevance in veterinary orthopedics (Durmuş et al, 2009). The genetic and economic value of the animal, its weight, the type and location of the fracture, and the veterinarian's experience are crucial factors in the treatment of fractures in farm animals (Arıcan et al, 2024; ElShafaey et al, 2014).
This study aim to assess extremities fractures in calves aged 0-6 months by clinical, thermographic, and radiographic methods.

MATERIALS and METHODS

This research was performed in accordance with the Afyon Kocatepe University Experimental Animals Local Ethics Committee (No: 88-19, protocol approval dated 17.09.2020).

Material

The study involved 26 calves aged 0-6 months, presented to the Afyon Kocatepe University Veterinary Faculty Surgery Department Large Animal Clinic from 2019 to 2020 with lameness complaints, subsequently diagnosed with fractures through clinical and radiological assessments. The control group comprised 7 healthy calves of the same age range. A complete blood count was conducted using a hemogram instrument (Human, Humancount-80, Wiesbaden, Germany). A centrifuge apparatus (Nüve, Nüve 300, Turkey) was employed to isolate the serum from the blood specimens. Analyses of serum biochemical parameters were conducted using a biochemistry instrument (Human Humastar-180, Wiesbaden, Germany). Radiographic assessments were conducted using an X-ray apparatus (ATS CMP 200 DR 1000 mA). A/P and M/L images were acquired using a CR device (Fujifilm CR IR 392, Turkey). Thermographic assessments were conducted on instances with suspected fractures using a thermography instrument (LW-EAA-STC-IOS SEEK). ELISA test kits were conducted using an ELISA reading instrument (Biotek Instruments, MWGt Lambda Scan 200, Winooski, VT, USA). The biochemical investigation utilized test kits from the YLBiont brand (Shanghai YL Biotech Co. Ltd., No. 5588, Caoan Road, Jiading District, Shanghai, China).

Method

Initially, clinical tests were conducted on calves with fractures of the metacarpus, metatarsus, femur, antebrachium, and tibia included in the thesis study. Thermographic assessments were conducted on suspected fracture cases using a thermography device (LW-EAA-STC-IOS SEEK Thermal Camera - IOS); alterations in the color scale and the resulting findings were documented, and the acquired images were archived. Radiographic examinations were conducted using the ATS CMP 200 DR 1000 mA X-ray equipment, obtaining A/P and M/L pictures, which were analyzed on the Fujifilm CR IR 392 CR device, and fracture cases were assessed. Incoming fracture patients were categorized and assessed. Based on the acquired findings, a diagnosis was established, and the corresponding treatment procedure was implemented accordingly. Hematological and biochemical analyses were conducted using blood samples obtained from the jugular veins of the calves.

Biochemical Measurements

Routine Biochemical Analyses

Blood collected in gel tubes devoid of anticoagulant was centrifuged at 5000 rpm for 3 minutes (Nüve, Nüve 300, Turkey), and the resultant serums were aliquoted and preserved at -20 °C until analysis. The serum samples were analyzed for creatinine, potassium, total protein, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, calcium, urea, magnesium, gamma-glutamyl transferase, and glucose parameters.

Assessment of Serum Concentrations of TNF-α, IL-1β, IL-6, Cortisol, TSH, T4, MDA (Malondialdehyde), and AOA (Antioxidant Activity) in Blood Serum

The following parameters were examined in these tests: serum TNF-Alpha (Bovine Tumor Necrosis Factor, TNF ELISA Kit, Catalog No: E0133b, BO), serum Interleukin 1β (IL-1β) (Bovine Interleukin-1IL-1β, ELISA Kit, Catalog No: YLA0051 BO), serum

Interleukin 6 (IL-6) (Bovine Interleukin-6 IL-6, ELISA Kit, Catalog No: E0079b, BO), serum Cortisol (Bovine cortisol ELISA kit, Catalog No: YLA0060BO), serum T4 (Bovine Thyroxine ELISA kit, Catalog No: YLA0079BO), and serum TSH (Bovine Thyroid Stimulating Hormone ELISA kit, Catalog No: YLA0132BO) receptor antagonist. Furthermore, malondialdehyde (MDA) and antioxidant activity (AOA) levels were analyzed in blood serum samples.

Assessment of MDA (Malondialdehyde) and AOA (Antioxidant Activity) Levels in Serum

The MDA level in samples was assessed using a twofold boiling method, which is a version of the Drapper and Hadley (1990) technique. During the initial boiling, MDA associated with the materials is liberated from the proteins, resulting in protein precipitation. In the subsequent boiling, the total MDA interacts with TBA, and the absorbance of the resulting colored complex is quantified at 532 nm. The concentration is determined using the molar absorption coefficient of MDA.

The usual Fe-EDTA complex solution reacts with hydrogen peroxide via the Fenton reaction, facilitating the generation of hydroxyl radicals. Reactive oxygen radicals breakdown benzoate, leading to the release of TBARS. The incorporation of antioxidants into human fluids results in the inhibition of TBARS formation. The reaction is quantified colorimetrically, with the inhibition of color development assessed as AOA (Koracevic et al., 2001).

Statistical Analysis

The findings derived from the research were analyzed utilizing One-Way ANOVA and the Student's T-test for paired groups within the SPSS 16.0 statistical software. The Duncan test was utilized for the results exhibiting statistical differences, and the data were presented as "Mean ± standard deviation." A P value of less than 0.05 was deemed statistically significant**.**

RESULTS

This study involved clinical, thermographic, and radiographic assessments of 18 metacarpal fractures, 3 femoral fractures, 3 metatarsal fractures, 1 antebrachial fracture, 1 tibial fracture, and 7 healthy cases in calves aged 0-6 years, presented at the surgical big animal clinic from 2019 to 2020.

The clinical examination results, comprehensive blood and biochemical analysis findings, radiographic examination results, and thermographic examination findings for 18 metacarpal, 3 femoral, 3 metatarsal, 1 antebrachial, and 1 tibial fractures are presented below.

Results of Clinical Examination

69.24% of the fractures involved the metacarpus, 11.53% the femur, 11.53% the metatarsus, 3.85% the tibia, and 3.85% the antebrachium. It was established that 84.62% of the fracture cases were attributable to dystocia, whereas 15.38% were due to various injuries occurring in the postnatal period. 61.54% of the fracture cases were male calves, while 38.46% involved female calves. It was established that 26.92% of the fracture cases were left-sided, 65.38% right-sided, and 7.7% bilateral. The average age of the fracture cases was established as 7.61±2.12 days, and the average weight was 45.38±3.53 kg. The average pulse rate for fracture cases is 123.07 ± 8.48 beats per minute, and the average respiratory rate is 51 ± 5.65 breaths per minute. In fracture cases, 76.92% of the mucosa is normal, 15.39% is pale, and 7.69% is hyperemic. In the assessment of lymph nodes in fracture cases, 88.46% were normal, 7.69% exhibited minor swelling, and 3.84% were swollen. In fracture instances, 76.92% exhibited normal hunger, while 23.04% experienced diminished appetite.

Results of Complete Blood Count

This study conducted statistical analyses of complete blood results from blood samples obtained from the jugular vein of both the control and fracture groups. Upon comparison of the control group with the fracture group, LYM (%), GRA (%, 109.l-1), Hb, RBC, MCV, and HCT values were statistically significant ($p<0.05$), although WBC, MID (%, 109.l-1), LYM (109.l-1), and PLT results were not significant ($p>0.05$) (Table 1).

Results of Serum Biochemistry Measurements

This study conducted statistical analysis of complete blood results from blood samples collected from the jugular vein of both the control and fracture groups. The comparison of the control group with the fracture group revealed statistically significant differences in CRE, TP, AST, Ca++, and GGT values ($p<0.05$), although K+, ALT, ALP, URE, Mg++, and GLU results were not statistically significant ($p>0.05$) (Table 2).

Biochemical tests revealed statistically significant differences ($p<0.05$) in the values of TNF-α (pg.ml-1), IL-1β (pg.ml-1), IL-6 (pg.ml-1), Cortisol (mcg.dl-1), MDA (µmol.l-1), and AOA (µmol.l-1) when comparing the control group to the fracture group. Table 3.

Table 1: Complete blood count results in the control and fracture groups

Group Parameters	Control	Fracture	Reference Range (Roadknight et al, 2021)	P
WBC (10^9.l-1)	9.88	12.86	4.0-21.2	0.255
LYM (%)	78.08	44.91	45-75	**0.000**
MID (%)	4.94	2.95	2.0-7.0	0.127
GRA (%)	17.00	52.11	15-65	**0.000**
LYM (10^9.l-1)	8.03	5.01	0.1-10.3	0.127
MID (10^9.l-1)	0.49	0.40	0.0-2.3	0.651
GRA (10^9.l-1)	1.36	7.44	0.6-6.70	**0.000**
Hb (g.dl-1)	10.61	8.76	6.4-15.1	**0.014**
RBC (10^{12}.l-1)	13.78	7.24	6.8-14.6	**0.0604**
MCV (fl)	30.31	35.53	26.5-44.5	**0.002**
HCT (%)	44.19	25.50	19-47	**0.026**
PLT (10^9.l-1)	264.71	277.00	161-1313	0.713

(WBC: White blood cells, LYM%: Lymphocyte percentage, MID%: Monocyte, GRA%: Granulocyte, LYM: Lymphocyte count, MID: Monocyte count, GRA: Granulocyte count, Hb: Hemoglobin, RBC: Red blood cells, MCV: Mean Erythrocyte Volume, HCT: Hematocrit percentage, PLT: Platelet count)

Table 2: Serum biochemistry results in the control and fracture groups

Group Parameters	Control	Fracture	Reference Range (Roadknight et al, 2021)	P
CRE (mg.dl-1)	1.01	2.02	0.5-2.2	**0.010**
K^+ (mEq.l-1)	4.81	5.12	4.2-7.2	0.132
TP (g.dl-1)	6.53	5.64	4.4-8.4	**0.018**
AST (U.l-1)	69.27	116.69	60-125	**0.034**
ALT (U.l-1)	23.70	22.65	6.8- 22.3	0.848
ALP (IU.ml-1)	257.85	373.50	123-738	0.183
Ca^{++} (mg.dl-1)	10.60	11.67	8-11.7	**0.006**
URE (mg.dl-1)	21.77	28.41	10-25	0.143
Mg^{++} (g.dl-1)	2.26	2.34	1.5-2.9	0.596
GGT (U.l-1)	29.42	659.53	26-1379	**0.000**
GLU (mmol.l-1)	89.41	90.85	40-100	0.822

(CRE: Creatinine, K+: Potassium, TP: Total protein, AST: Aspartate Aminotransferase test, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, Ca++: Calcium, URE: Urea, Mg++: Magnesium, GGT: Gamma Glutamine Transtera, GLU: Glucose)

Table 3: ELISA results in the control and fracture groups

Parametres	Control	Fracture	P
TNF-α **(**pg.ml-1)	0.11	0.15	**0.002**
IL-1β **(**pg.ml-1)	18.67	30.69	**0.000**
IL-6 **(**pg.ml-1)	61. 79	98.29	**0.000**
Cortizol (mcg.dl-1)	3.36	4.93	**0.000**
MDA (µmol.l-1)	3.77	5.00	**0.000**
AOA (µmol.l-1)	6.80	4.95	**0.000**

(TNF-α: Tumor necrosis factor-alpha, IL-1β: Interleukin-1 beta, IL-6: Interleukin-6, MDA: Malondialdehyde, AOA: Antioxidant activity)

Findings from Radiographic Examination

Based on the relationship between the fracture ends and the external environment, 88.47% of the cases are classified as closed fractures, whereas 11.53% are categorized as open fractures. Based on the quantity of fracture fragments, 73.08% are classified as two-fragmented, whereas 26.92% are categorized as comminuted fractures. 42.3% of fracture cases are classified as displaced fractures, whereas 57.7% are categorized as non-displaced fractures.

Based on the anatomical position of the fracture instances, 96.15% are diaphyseal, while 3.85% are epiphyseal. Diaphyseal fractures occur in 3.85% of the proximal one-third and 96.15% in the distal one-third. Based on the fracture line, 52% of diaphyseal fractures are transverse, whereas 48% are short oblique fractures. 69.23% of transversal fractures and 11.66% of short oblique fractures exhibited displacement (Table 4).

Results of Thermographic Examination

Thermographic and body temperature assessments of the animals in both the control and fracture groups were conducted. The thermographic assessments conducted in the fracture region of the fracture group yielded an average temperature of 30.85±4.33 °C, while the average body temperature was 37.92±0.83 °C. The comparison of thermographic measures with body temperature yielded statistically significant results ($p<0.05$). The control group had an average temperature of 26.71±2.13 °C, while the average body temperature was recorded at 38.47±0.44 °C. The comparison of thermographic measures with body temperature yielded statistically significant results ($p<0.05$) (Table 5).

In the fracture group, the mean temperature was 30.85±4.33 °C in the thermographic assessments conducted in the fracture region, while in the control group, the mean temperature was 26.71±2.13 °C. The thermographic temperature disparity between the fracture group and the control group was shown to be statistically significant ($p<0.05$) (Table 5).

Table 4: Radiographic findings of the fracture group

Case	Bone	Side	Localization
C1	Tibia	Right	Distal 1/3 transversal displaced
C2	Metacarpus	Bilateral	Distal 1/3 short oblique
C3	Metacarpus	Right	Distal 1/3 short oblique
C4	Metatarsus	Left	Distal 1/3 short oblique
C5	Metacarpus	Right	Salter Harris Tip II
C6	Femur	Right	Distal 1/3 transversal displaced
C7	Metacarpus	Right	Distal 1/3 kısa oblik deplase fragmentary
C8	Metatarsus	Left	Proksimal 1/3 short oblique displaced
C9	Metacarpus	Left	Distal 1/3 transversal
C10	Metacarpus	Right	Distal 1/3 short oblique
C11	Metacarpus	Left	Distal 1/3 short oblique
C12	Metacarpus	Right	Distal 1/3 short oblique fragmentary
C13	Metatarsus	Right	Distal 1/3 transversal fragmentary
C14	Antebrachium	Right	Distal 1/3 transversal displaced
C15	Metacarpus	Right	Distal 1/3 short oblique
C16	Metacarpus	Bilateral	Distal 1/3 transversal displaced
C17	Metacarpus	Right	Distal 1/3 short oblique
C18	Femur	Left	Distal 1/3 transversal displaced
C19	Femur	Right	Distal 1/3 transversal displaced
C20	Metacarpus	Right	Distal 1/3 transversal
C21	Metacarpus	Left	Distal 1/3 short oblique
C22	Metacarpus	Right	Distal 1/3 transversal
C23	Metacarpus	Right	Distal 1/3 transversal displaced
C24	Metacarpus	Right	Distal 1/3 short oblique
C25	Metacarpus	Right	Distal 1/3 transversal displaced
C26	Metacarpus	Left	Distal 1/3 transversal displaced

Table 5: Thermography and Body Temperature Averages

Group	Thermography	Body Temperature	p
Fracture(°C)	30.85	37.92	0.000
Control (°C)	26.71	38.47	0.000
p	0.024	0.112	

DISCUSSION

Görgül et al. (2004) indicated that 93.5% of fracture cases in calves were Holstein and 6.5% were Simmental, however Gangl et al. (2006) showed that 90.91% were Belgian Blue/White, 6.06% were dairy, 2.02% were Blonde d'Aquitaine, and 1.01% were Charolais. Yanmaz et al. (2014) determined that 52.3% of fractures occurred in crossbreds, 20% in Brown Swiss, 18.5% in East Anatolian Red (EAR), 4.6% in Holstein, and 4.6% in Simmental. This study found that 73.08% of the fracture instances involved Simmental cattle, while 26.92% involved Holstein cattle. This rate is attributed to the increased feeding of Simmental breed livestock in the Afyonkarahisar region.

Gangl et al. (2006) reported that 58.59% of the fracture cases in calves were in the change and 41.41% were in males. However, Arıcan et al. (2024) reported that 80% of the fracture cases were in females and 20% were in female calves. In addition, Alam et al. (2014) reported that 66.67% of the fracture cases in calves were in males and 33.33% were in females.Akın (2017) found that 65.62% were male, attributing the increased fracture susceptibility in male calves to dystocia resulting from their greater birth weight compared to females. This study found that, consistent with existing literature, 61.54% of fracture cases were male and 38.46% were female.

Aksoy et al. (2009) indicated that the ages of calves with limb fractures varied from 1 to 30 days, with the majority being 1 to 3 days old. Feist et al. (2019) observed that 67.5% of extremities fractures in calves occurred in individuals younger than two weeks, with 60.8% occurring during parturition or postnatal handling. This study found that fractures occurred in calves with an average age of 7.61±2.12 days. The results are consistent with the existing literature.

Fractures during birth predominantly occur in the metacarpal/metatarsal regions, as birth aids are typically utilized in these areas (Aksoy et al, 2009; Yanmaz et al, 2014). Numerous researches have indicated that improper or excessive traction during challenging deliveries predominantly results in traumatic injuries to the muscles, bones, joints, nerves, and other soft tissues in the metacarpus and metatarsus (Akın, 2017). Arıcan et al. (2014) highlighted the etiology of fracture cases in their study, indicating that 32.5% of calf fractures resulted from excessive force and improper help during parturition, whilst the remaining 67.4% were attributed to trauma and inadequate treatment. Yanmaz et al. (2014) indicated that 20 fracture cases (30.8%) resulted from disproportionate and inadvertent force applications during delivery, whereas 45 cases (69.2%) were attributed to traumatic incidents such as slipping, falling, and impact. In the fracture cases included in this study, 84.62% were attributed to dystocia, while 15.38% resulted from various traumas during the postpartum period. This outcome aligns with the conclusions of Arıcan et al. (2014) and Yanmaz et al. (2014).

The inflammatory phase of normal bone healing occurs within the initial 24-48 hours. It persists until the development of new blood vessels, granulation tissue, and cartilage. Currently, the fracture site is predominantly infiltrated by polymorphonuclear leukocytes, macrophages, and mast cells (Paskalev, 2009). This study detected an elevation in WBC numbers in the fracture group (12.86±1.69 10^9.l^-1) compared to the control group (9.88±1.87 10^9.l^-1), although this difference was not statistically significant ($p>0.05$).

The elevation in creatinine levels may result from muscular injury and physical exertion. Reductions in creatinine levels may result from diminished creatine synthesis in the liver and a sluggish metabolism in muscle tissue. Nikolaevna and Uygunovich (2021) documented in their study involving rabbits with experimental fractures that serum creatinine levels increased arithmetically on the 7th day relative to the 0th day and subsequently decreased by the 90th day, attributing this variation to the equilibrium between creatinine synthesis and metabolism. In this investigation, the creatinine level of the control group animals was measured at 1.01±0.08 mg.dl-1, which was found to be comparable to the findings of Rassel et al. (2021) in calves (1.303±0.0009). The creatinine level in fracture cases was significantly elevated compared to the control group (2.02±0.35 mg.dl-1) with statistical significance ($p<0.05$). The elevation of creatinine levels in the fracture group is believed to stem from heightened protein catabolism due to soft tissue injury.

The activity of transaminases is utilized to assess the extent of impairment in the activities of the liver, heart, and skeletal muscles. The elevation of these enzymes in blood serum correlates with their release from injured organs and tissues (Nikolaevna and Uygunovich, 2021).

Serum AST levels elevate in cases of skeletal muscle injury. Pearson et al. (2019) assessed AST levels in calves subjected to trauma from dystocia and found an elevation in blood AST levels. This study found AST levels to be 116.69±20.34 U.l-1 in fracture patients and 69.27±6.40 U.l-1 in the control group, with this increase being statistically significant ($p<0.05$). The elevated serum AST levels in calves with fractures, in comparison to the control group, may result from damage to adjacent soft tissues, and this finding aligns with existing research.

Glutamyl transferase (GGT) serves as a marker for colostrum consumption in calves (Wolf et al, 2021). GGT levels have been seen to rise in cases of hepatocellular and biliary tract damage (Clark et al, 1987). In this investigation, serum GGT levels were measured at 29.42±11.98 U.l-1 in the control group and 659.53±151.76 U.L-1 in the fracture group, both falling within reference ranges. Despite the GGT value

being statistically considerably elevated in the fracture group ($p<0.05$), it lacks clinical significance.

The concentration of Serum Total Protein (TP) in neonatal calves is approximately 4-7 g/dl (Wood and Quiroz-Rocha, 2010). In their investigation on calves with femur fractures, Bellon and Mulon (2011) reported an average blood total protein level of 5.57 g.dl-1. Steiner et al. (1996) assessed the total protein level in a calf with a metacarpal fracture and reported that it fell within the reference range. In this investigation, serum total protein concentrations were measured at 6.53±0.26 g.dl-1 in the control group and 5.64±0.19 g.dl-1 in fracture cases, both falling within the reference range. Despite a statistically significant difference in TP levels across the groups ($p<0.05$), the difference lacked clinical significance.

Antioxidant activity (AOA) denotes the overall antioxidant capacity of blood (Chirase et al., 2004). The heightened generation of reactive species may lead to a reduction in in-vivo antioxidant activity values (Aengwanich et al., 2011). Chirase et al. (2004) showed a decline in AOA levels in calves subjected to transportation stress. In this investigation, serum AOA levels were quantified at 6.80±0.64 µmol.l-1 in the control group and 4.95±0.92 µmol.l-1 in fracture cases, demonstrating statistical significance ($p<0.05$). The reduced AOA values in fracture cases relative to the control group are attributable to oxidative stress. These findings align with the existing research.

Sheweita and Khoshhal (2007) indicated that ALP levels rose subsequent to fracture development. This investigation revealed elevated ALP levels in the fracture group (373.50±76.38 IU.ml-1) relative to the control group (257.85±37.20 IU.ml-1); however, the difference was not statistically significant ($p>0.05$). Conversely, the ALP level in the fracture group rose by almost 50% compared to the control group. This corroborates the literature.

Bone tissue markers are utilized to assess the impacts on bone production, osteoporosis, or both conditions. Calcium serves as a marker for bone loss, whereas alkaline phosphatase (ALP) indicates bone growth and osteoblastic activity (Bozukluhan et al, 2018). Calcium is essential for the mineralization of the callus, vital to the fracture healing process. As dietary calcium is insufficient to fulfill this requirement, the calcium necessary for callus mineralization is predominantly sourced from bone tissue, which serves as a calcium reservoir, to facilitate proper bone healing (Fischer et al, 2018). Chaurasia et al. (2019) revealed in their study on dogs that serum calcium levels diminished on the 15th postoperative day, attributing this decline to excessive calcium deposition at the fracture site. Furthermore, Mohuiddin et al. (2018) assessed the blood calcium levels in calves on the day of fracture (day 0) and again on the 21st day, noting that the calcium level was normal on day 0 but diminished by day 21. The reduction in serum calcium levels may be attributed to increased calcium mobilization during callus development and accelerated healing (Deka et al., 1994). This investigation determined the calcium level in the fracture group to be (11.67±0.23 mg.dl-1), which was significantly higher than the control group (10.60±0.25 mg.dl-1), with statistical significance ($p<0.05$). Despite the statistical significance of this variation, it lacks clinical relevance as the calcium measurements remain within the reference range.

The acute phase response is a multifaceted systemic defensive mechanism activated by trauma, infection, stress, neoplasia, and inflammation (Cray et al, 2009). The three proinflammatory cytokines, TNF-α, IL-1β, and IL-6, are regarded as the primary activators of the systemic inflammatory response. These cytokines serve as the primary mediators of the acute phase response. Inflammation, infection, or tissue injury prompts cytokine secretion by immune cells. Consequently, the synthesis of acute phase proteins is stimulated. TNF-α is a significant mediator of osteopenia (Sheweita and Khoshhal, 2007). TNF-α concentration reportedly peaks within the initial 24 hours post-trauma and diminishes after 72 hours (Gerstenfeld et al, 2003). TNF-α is synthesized by macrophages and other inflammatory cells, functioning as a chemotactic agent by eliciting secondary inflammatory signals. It is also recognized for inducing the development of mesenchymal stem cells (MSCs) into osteogenic cells in vitro (Marsell and Einhorn, 2011). IL-1β and IL-6 are particularly crucial for the process of fracture healing. IL-1β expression coincides with that of TNF-α in a biphasic manner. Macrophages create it during the acute phase of inflammation, and osteoblasts also stimulate IL-6 production. They facilitate the formation of primary cartilaginous callus. They promote angiogenesis, induce vascular endothelial growth factor (VEGF) production, and contribute to the development of osteoblasts and osteoclasts (Marsell and Einhorn, 2011). It is well acknowledged that physical and psychological stress elevates plasma IL-6 and acute phase protein concentrations in people and experimental animals. Evidence exists that physical stress can stimulate the production of acute phase proteins in cattle (Murata et al, 2004). Korkmaz et al. (2015) showed in their study that TNF-α, IL-1β, and IL-6 concentrations increased 15 minutes post-cauterization operation. The investigation revealed that concentrations of TNF-α, IL-1β, and IL-6 were significantly elevated in fracture patients compared to the control group ($p<0.05$). The elevation of inflammatory mediators during the acute phase of fractures aligns with existing studies.

The assessment of alterations in blood cortisol levels is typically utilized as a marker for the immediate response to stress. Korkmaz et al. (2015) indicated that the cortisol levels in calves with cauterized horns elevated. Our investigation revealed that cortisol levels in fracture cases (4.93±0.97 mcg.dl-1) were statistically substantially elevated compared to the control group (3.36±0.54 mcg.dl-1) ($p<0.05$). The elevation of serum cortisol levels correlates with the fracture occurrence

resulting from the trauma's intensity. Fractures arise following significant mechanical trauma that concurrently inflicts harm on adjacent soft tissues. To rectify this damage, both local and systemic responses are initiated, evidenced by the activation of cellular and humoral components (Keel and Trentz 2005). Numerous studies have indicated a correlation between bone healing and elevated reactive oxygen species (ROS) in the fracture site. Lipid peroxidation byproducts serve as dependable markers of oxidative stress at the fracture site, in blood serum, and in urine (Paskalev, 2009). Following a bone fracture, the generation and buildup of reactive oxygen species (ROS) at the fracture site and in the peripheral circulation arise due to the disruption of blood flow at the fracture ends. This is a localized indicator of oxidative stress that impacts the entire organism and can significantly modify the bone healing process (Paskalev, 2009). Oxidative stress has been shown in both spontaneous and artificially induced fractures in rats (Yeler et al., 2005) as well as in experimental osteotomies conducted on rats (Tasatargil et al., 2007). Trauma stimulates the hypothalamic-pituitary-adrenal axis, the primary mechanism for stress response (Pacák and Palkovits, 2001). Cytokines are chemicals released by the pituitary gland that stimulate the adrenal cortex to respond to stress (John and Buckingham, 2003). Diverse traumas can induce oxidative stress by the enhancement of lipid peroxidation and oxidation or by eliciting an inflammatory response in the tissue (Baines and Shenkin, 2002). Lipid peroxidation and plasma MDA concentrations are directly correlated with the extent of tissue damage (Prasad et al, 2003). Korkmaz et al. (2015) documented an elevation in MDA levels in calves subjected to surgery in their study. The investigation revealed that MDA levels were elevated in calves with fractures (5±0.73 μmol.l-1) compared to the control group (3.77±0.39 μmol.l-1), with statistical significance ($p<0.05$). This outcome parallels that of Korkmaz et al. (2015). Fractures of the extremities in calves comprise 21-50% metacarpal and metatarsal injuries, followed by 15-32% femoral fractures and 12-15% tibial fractures (Gangl et al, 2006). Arıcan et al. (2014) indicated that fractures in calves predominantly occurred in the metacarpal bones (60.6%), followed by the femur (14.9%), metatarsal bones (7.1%), tibia (8.8%), antebrachium (6%), and humerus (3.2%). Mohuiddin et al. (2018) documented fractures as follows: 40% metacarpal, 20% metatarsal, 20% radial, 10% humeral, and 10% femoral. This study revealed that 69.24% of fracture cases in calves involved the metacarpus, 11.53% the femur, 11.53% the metatarsus, 3.85% the tibia, and 3.85% the antebrachium. The findings were found to be consistent with the literature.

Research indicates that the anatomical distribution of fracture lines comprises 36.36% distal epiphyseal, 63.64% diaphyseal (Tulleners, 1986), 84.5% distal diaphyseal, 10.4% proximal diaphyseal, 2.2% epiphyseal, 1.6% metaphyseal, and 1.1% comminuted fractures (Arıcan et al, 2014), along with 60.86% diaphyseal, 28.98% metaphyseal, 5.80% distal epiphyseal, and 4.36% metaphyseal fractures with epiphyseal separation (Belge et al, 2016). Akın (2017) discovered that the predominant occurrence of calf extremity fractures (68.75%) takes place at the distal diaphysis in a study. This study found that 96.15% of fractures were diaphyseal, whereas 3.85% were epiphyseal, based on the anatomical placement of the fracture line. It was established that 3.85% of the diaphyseal fractures occurred in the proximal one-third, whereas 96.15% were located in the distal one-third. The findings were found to be consistent with the existing literature. Belge et al. (2016) indicated that all fracture instances were closed in their research. Tullener (1986) indicated that 69.70% of fracture cases were closed and 30.30% were open fractures in his study, but Mohuiddin et al. (2018) found that 70% were closed and 30% were open fractures. In our study, 88.47% of the fractures were classified as closed, while 11.53% were classified as open, based on the relationship of the fracture ends to the external environment. 42.3% of the fracture cases were classified as displaced fractures, whereas 57.7% were categorized as non-displaced fractures. The results are incongruent with Belge et al. (2016), although analogous to Tullener (1986) and Mohuiddin et al. (2018).

Inflammation leads to heightened circulation in the affected area, resulting in an elevation of temperature as a primary symptom. Any trauma or illness invariably induces alterations in circulation. Thermography identifies a "hot spot" linked to localized inflammation (Redaelli et al, 2014). A 1 °C discrepancy between two anatomically symmetrical regions signifies inflammation in that location. Yanmaz et al. (2007); Soroko et al. (2013); Alsaaod et al. (2015). Thermography may serve as an adjunctive diagnostic method for identifying lameness in cattle. The assessment of local temperature variation is a crucial signal for the early identification of inflammation linked to lameness in inflammatory diseases (Alsaaod and Buscher, 2012). Redaelli et al. (2009) conducted thermographic assessments in both healthy and lame cows, establishing a correlation between thermography and the diagnosis of foot ailments. Whay et al. (2004) assessed the temperature of the metatarsal joint, lateral hoof wall, mid-tarsus, and lateral capsule ungula in the abaxial direction, concluding that lesioned feet exhibited higher temperatures than healthy feet. Renn et al. (2014) indicated that temperature was elevated in lame cows by thermography and proposed that thermography could serve as an alternate diagnostic instrument for lameness assessment. Alsaaod and Buscher (2012) indicated an elevation in the surface temperature of the coronary band of the affected foot relative to the healthy contralateral foot in lame cattle. Furthermore, the researchers determined that the disparities observed between healthy and lesioned feet, as assessed by thermography, could facilitate the

identification of hoof lesions in dairy cows without necessitating a clinical foot examination.

Cockcroft et al. (2000) employed thermography to detect septic arthritis of the metatarsophalangeal joint in a two-year-old Friesian heifer. They observed elevated temperatures in all lateral, medial, plantar, and dorsal projections of the inflamed metatarsophalangeal joint compared to the healthy contralateral joint, concluding that thermography can serve as a tool for localizing the inflamed area.

Stromberg (1974) validated the efficacy of thermography in identifying pathological alterations in the superficial digital flexor tendon (SDFT) prior to the manifestation of clinical inflammatory symptoms.

Vaden et al. (1980) employed thermography to identify subclinical arthritis. Doğan et al. (2016) employed thermography to diagnose septic arthritis in calves, identifying hot patches in affected joints.

Turner (1991) documented the application of continuous temperature monitoring in the foot for diagnosing subclinical inflammation up to two weeks prior to the manifestation of clinical lameness symptoms. The quality of thermograms is influenced by exercise, perspiration, body posture and angle, body coverings, systemic and topical pharmaceuticals, regional and local anesthetic blocks, sedatives, tranquilizers, anesthetics, vasoactive agents, and skin lesions such as scars (Alsaaod et al, 2015). No pharmacological intervention was administered in this study that could influence the outcomes.

This study revealed an elevation in temperature near the fracture line and its vicinity when juxtaposed with the control group. Thermographic investigations in the fracture group revealed an average temperature of 30.85±4.33 °C, whereas the control group exhibited an average temperature of 26.71±2.13 °C. The thermographic temperature disparity between the fracture group and the control group was determined to be statistically significant ($p<0.05$). The temperature increase was determined to be associated with the inflammatory phase, the initial stage of fracture healing. The literature analysis revealed an absence of evidence concerning the application of thermography in fracture cases involving calves. Consequently, thermographic assessment may be utilized to monitor the inflammatory process in fracture situations.

CONCLUSION

Fracture cases resulting from dystocia and trauma in calves hold significant relevance among surgical disorders affecting calves. The conclusion was reached that thermography can serve as a diagnostic tool in fracture cases, and that implementing the appropriate treatment option will significantly benefit the national economy. Furthermore, more comprehensive studies are required regarding the application of thermographic examination in the postoperative period alongside fracture diagnosis.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: YK and ZKS contributed to the project idea, design and execution of the study. YK and ZKS contributed to the acquisition of data. YK and ZKS analysed the data. YK and ZKS drafted and wrote the manuscript. YK and ZKS reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Ethical approval: This study was carried out at Afyon Kocatepe University Veterinary Health Application and Research Center. This research was approved by The Ethics Committee of the Faculty of Veterinary Medicine, Afyon Kocatepe University (AKUHADYEK, Ref No: 109, Tarih: 18/09/2019)

Explanation: We have presented as a (oral) at the 17th National Veterinary Surgery Congress ve 3nd International Veterinary Surgery Congress of Turkey. (2022). This study was prepared from a master thesis.

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RESEARCH ARTICLE

Echocardiographic Characteristics and Main Pulmonary Artery/Aorta Ratio of Dogs with Tracheabronchitis

Gencay EKİNCİ[1]*, Celil BENDEŞ[2]

[1]Erciyes Üniversitesi, Veterinerlik Fakültesi, İç Hastalıkları Anabilim Dalı, 38280, Kayseri/ Türkiye
[2]Erciyes Üniversitesi, Sağlık Bilimleri Enstitüsü, Veterinerlik İç Hastalıkları Anabilim Dalı, 38280, Kayseri/ Türkiye

ABSTRACT

An acute or chronic inflammation of the trachea and bronchial airways, which can spread to the lungs, is called tracheobronchitis. Monitoring basic echocardiographic parameters in dogs with tracheobronchitis may be useful in detecting early cardiac signs of pulmonary hypertension (PH). Pulmonary artery (PA) dilation is one of the cardiac abnormalities associated with pulmonary hypertension. This study aimed to main pulmonary artery diameter and main pulmonary artery (MPA)/aorta (Ao) ratio in dogs with tracheobronchitis. The animal material of the study consisted of 90 dogs with tracheobronchitis aged 1-14 years (mean 7±3 years), mostly small breeds (Terrier, Pomeranian, Russian Poodle, et al.). Two-dimensional, M-mode and pulse wave Doppler techniques were used in echocardiographic examination. The diameters of the MPA and Ao were measured in the right parasternal short axis view and the MPA/Ao ratio was calculated. The mean MPA diameter, Ao diameter and MPA/Ao ratio of dogs with tracheobronchitis were calculated as 1.20±0.31 cm, 1.37±0.36 cm and 0.90±0.16, respectively. There was a strong positive correlation between body weight and pulmonary artery diameter ($r=0.754$, $P<0.001$). As a result, the MPA/Ao ratio in dogs with tracheobronchitis included in the study was within the values reported for healthy dogs. Based on basic echocardiographic findings and pulmonary artery measurements, no signs of pulmonary hypertension were detected in dogs with tracheobronchitis. However, it can be argued that due to the progressive nature of PH and its poor prognosis, a more comprehensive echocardiographic evaluation may be useful to identify dogs with the potential to develop PH.
Keywords: Aorta, Dog, Echocardiography, Main pulmonary artery, Tracheobronchitis

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Trakeabronşitli Köpeklerin Ekokardiyografik Özellikleri ve Ana Pulmoner Arter/Aorta Oranı

ÖZ

Trakeobronşit trakea ve bronşiyal hava yollarının akut veya kronik yangısına denir. Pnömoni, trakeobronşiyal hastalık ve infiltratif pulmoner hastalık gibi birçok solunum yolu hastalığı köpeklerde pulmoner hipertansiyon ile ilişkilendirilmiştir. Trakeobronşitli köpeklerde temel ekokardiyografik parametrelerin izlenmesi, pulmoner hipertansiyonun (PH) erken kardiyak belirtilerinin tespit edilmesinde faydalı olabilir. Pulmoner arter (PA) dilatasyonu, pulmoner hipertansiyon ile ilişkili kardiyak anormalliklerden biridir. Bu çalışmada trakeobronşitli köpeklerde ana pulmoner arter çapı ve ana pulmoner arter (MPA)/aort (Ao) oranının belirlenmesi amaçlanmıştır. Çalışmanın hayvan materyalini Terrier, Pomeranian, Russian Poodle, Pekingese, Pug gibi çoğunlukla küçük ırklardan oluşan 1-14 yaş arası (ortalama 7±3 yaş) toplam 90 trakeobronşitli köpek oluşturdu. Ekokardiyografik muayanede iki boyutlu (2D), M-mod ve PW Doppler inceleme teknikleri kullanıldı. MPA ve Ao çapları sağ parasternal kısa eksen görünümünde ölçüldü ve MPA/Ao oranı hesaplandı. Trakeobronşitli köpeklerin ortalama MPA çapı, Ao çapı ve MPA/Ao oranı sırasıyla 1.20±0.31 cm, 1.37±0.36 cm ve 0.90±0.16 olarak hesaplandı. Canlı ağırlı ile pulmoner arter çapı arasında güçlü bir pozitif korelasyon vardı ($r=0.754$, $P<0.001$). Sonuç olarak, çalışmaya dahil edilen trakeobronşitli köpeklerde MPA/Ao oranı sağlıklı köpekler için bildirilen referans değerler arasındaydı. Temel ekokardiyografik bulgulara ve pulmoner arter ölçümlerine dayanarak, trakeobronşitli köpeklerde pulmoner hipertansiyon belirtisi tespit edilmemiştir. Bununla birlikte, PH'nin progresif yapısı ve kötü prognozu nedeniyle, PH gelişme potansiyeli olan köpekleri belirlemek için daha kapsamlı bir ekokardiyografik değerlendirmenin yararlı olabileceği söylenebilir.
Anahtar Kelimeler: Ana pulmoner arter, Aorta, Ekokardiyografi, Köpek, Trakeobronşitis

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ORCID ID; *GE: 0000-0002-4551-8749, CB: 0009-0008-5777-1443*
*Corresponding author e-mail: gekinci@erciyes.edu.tr

INTRODUCTION

An acute or chronic inflammation of the trachea and bronchial airways, which can spread to the lungs, is called tracheobronchitis. It frequently manifests in dogs already afflicted by respiratory diseases or disorders of the lungs or airways (Rozanski et al. 2020). Infectious tracheobronchitis frequently accompanies a viral infection of the respiratory system (Maden et al. 2000; Reagan and Sykes 2020). Other causes of tracheobronchitis in dogs include parasites, oral and pharyngeal disorders, chronic coughing due to heart or lung disease, smoke inhalation, and chemical fume exposure (Kumrow and Rozanski 2012). Chronic bronchitis most commonly affects small breed dogs, but it can also occur in large breeds. Chronic bronchitis in middle-aged and older dogs may worsen in response to rapid weather changes or other environmental pressures (MSD Vet Manuel). Chronic bronchitis is defined as a cough that lasts at least two months and is not caused by another respiratory disease. The most noticeable symptom is coughing spasms. The acute stage of bronchitis lasts two to three days, although the cough might last for weeks. The disease is diagnosed based on a history, physical examination, clinical symptoms, and the exclusion of alternative causes of coughing (Kumrow and Rozanski 2012). Dogs with tracheobronchitis usually have a loud cough that sounds like a 'goose honk'. Other signs of tracheobronchitis include coughing when the dog's throat is rubbed, coughing during or after exercise, gagging, spitting up frothy saliva, runny eyes and nose, swollen tonsils, wheezing, loss of appetite, depressed or lethargic behavior (Day et al. 2020). In mild cases or those with recent start of symptoms, supportive care may be useful; nevertheless, treatment of the underlying disease (if present) is also required. Rest, warmth, and good hygiene are all crucial. Corticosteroids are commonly used to reduce airway inflammation in dogs with chronic bronchitis. If a bacterial infection exists, antibiotics may be provided (Vieson et al. 2012).

In small animal practice, echocardiography is the most effective diagnostic tool for assessing heart anatomy, function, and cardiovascular illness (Bonagura and Miller 1989, Vurucu et al. 2021; Terzi, 2022). The pulmonary artery (PA) is a key site for assessing pulmonary hypertension (PH) in dogs using echocardiography, as recommended by the American College of Veterinary Internal Medicine (ACVIM) guidelines (Reinero et al. 2020). Echocardiographic assessment of pulmonary hypertension (PH) is primarily based on typical cardiac alterations (i.e., echocardiographic signs of PH) and the estimation of pulmonary arterial pressure (PAP) from spectral Doppler tracings. Because right cardiac catheterization is rarely used for definitive diagnosis of pulmonary hypertension in dogs, veterinarians rely on echocardiography to diagnose, classify, and manage dogs with PH (Reinero et al. 2020). Parameters such as pulmonary artery (PA) diameter (PA/Ao>1.017), pulmonary artery pressure (PAP ≥25 mm Hg), right ventricle/left ventricle basal diameter ratio, peak early diastolic pulmonary regurgitation velocity (>2.5 m/s), flattening of the interventricular septum are used to help grade the probability of pulmonary hypertension (Augustine et al. 2018; Reinero et al. 2020; Grosso et al. 2023). Estimated systolic pulmonary artery pressure is most frequently used to classify the pulmonary hypertension as mild (30-55 mm Hg), moderate (55-80 mm Hg), or severe (>80 mm Hg) (Pyle et al., 2004; Campbell 2007).

Chronic obstructive pulmonary disease is the most common cause of pulmonary hypertension in humans (Olsson et al. 2023). Pneumonia, tracheobronchial disease, infiltrative pulmonary disease, laryngeal paralysis, pulmonary thromboembolism, *Angiostrongylus vasorum* infestation, interstitial pulmonary fibrosis of West Highland White Terriers, neoplasia, and *Dirofilaria immitis* have all been linked to pulmonary hypertension (Nicolle et al. 2006; Schober et al. 2006; Reinero et al. 2020). Pulmonary hypertension is a serious complication of pulmonary diseases, and severe hypertension has a poor prognosis (Campbell 2007). PA diameter, Ao diameter and PA/Ao ratio are basic parameters that can be measured quickly in echocardiographic examination. Dogs with tracheabronchitis may have increased main pulmonary artery (MPA) diameter and main pulmonary artery (MPA)-to-aorta (Ao) ratio. Dilatation of the MPA in dogs may be a normal anatomical variant or a sign of underlying cardiac or pulmonary disease. Chronic pressure increase in the pulmonary circulation causes dilatation of the MPA. It can be caused by left heart disease (mitral valve disease), pulmonary thromboembolism, chronic lung disease or heartworm disease. There are not many studies determining the ranges of these parameters in dogs with tracheobronchitis. Monitoring basic echocardiographic parameters (MPA diameter, Ao diameter, MPA/Ao ratio) may allow early diagnosis of these diseases or predisposition to them. This study aimed to define basic echocardiographic measurements, primarily pulmonary artery (PA) diameter and main pulmonary artery MPA/Ao ratio in dogs with tracheobronchitis.

MATERIALS and METHODS

Animals

The animal material of the study consisted of 90 dogs aged 1-14 years (median 4), mostly small breeds such as Terrier, Pomerian, Russian Poodle, Pekingese and Pug, which were brought to Erciyes University, Faculty of Veterinary Medicine, Veterinary Teaching Hospital, Small Animal Clinic.

Physical Examination

Each dog included in the study had a comprehensive physical assessment. These examinations were performed by a single individual. The dogs included in the study were subjected to a complete physical examination including examination of the mucous membranes, determination of capillary refill time, estimation of the degree of dehydration, palpation of the lymph nodes, body temperature (°C), pulse frequency (bpm), respiratory rate, auscultation of the lungs and heart (presence of murmurs, arrhythmias, etc.). Skin elasticity, capillary refill time, and mucous membrane dryness were used to evaluate dehydration levels (<5% [subclinical], 5% [mild], 6%-8% [moderate], 8%-10% [severe], and ~12% [hypovolemia]) (Tello et al. 2017).

Diagnosis of Tracheobronchitis, Inclusion and Exclusion Criteria

The diagnosis of tracheobronchitis in dogs was based on anamnesis, physical examination findings, diagnostic imaging (thoracic radiography) and laboratory (CBC) findings.

Inclusion criteria were clinical signs (presence of a harsh, dry, unproductive cough, usually worsened by tracheal palpation) of tracheobronchitis, cough lasting at least 2-3 days, runny nose, sneezing or gagging after coughing, noisy breathing or mild inspiratory stridor, normal or mild abnormalities on chest X-ray (to rule out pneumonia).

Dogs with pneumonia (productive cough, fever, significant radiographic lung involvement), tracheal collapse, concurrent systemic disease (e.g., severe cardiac disease, immunosuppression, or neoplasia), recent antibiotic, steroid, or immunosuppressive therapy were excluded.

Blood Pressure Measuring

Non-invasive blood pressure measures were taken with the PetTrust oscillometer (BioCARE, Taiwan). To reduce stress during blood pressure measures in dogs and help them adjust to unfamiliar persons, a 5-10 minute waiting interval was used. During the blood pressure readings, the animal owner, assistance staff, and the veterinarian doing the assessment were all present. The cuff size was determined using the measuring tape provided by PetTrust. To determine the proper cuff size, the circumference of the dog's right forelimb (proximal) was measured using a flexible measuring tape. The suitable cuff size was determined by measuring approximately 30% of the circumference of the right forelimb. To measure blood pressure in the proximal region of the right forelimb, the bladder of the cuff was put over the arteria radialis at the center of the antebrachium, making sure it wasn't too tight or too loose. When the device was activated, the cuff inflated automatically. Mean arterial pressure (MAP), diastolic blood pressure (DBP) and systolic blood pressure (SBP) were measured. The data was then recorded. All measurements were taken by an experienced veterinarian. The first blood pressure readings were dismissed. Following that, three measures were collected, with a 15-second delay between each reading. Data analysis was performed using the arithmetic average of these three readings.

Blood Sampling and Complete Blood Count Analysis

Blood samples were taken from the dogs' cephalic veins for hematological exams to detect infection status and leukocyte counts in accordance with clinic standard methods. Blood samples were inserted into BD Vacutainer® K_2 EDTA tubes (Becton Dickinson, USA) for hematological analysis. Hematological analyses were performed using a complete blood count device (Exigo EosVet, Boule Medical AB, Stockholm, Sweden).

Echocardiographic Examination

M-mode and 2D measurements were recorded according to the recommendations of American Society of Echocardiography and methodology published in the veterinary literature (Thomas et al. 1993). Echocardiographic examinations were performed using a multifrequency (3-11 MHz) Doppler ultrasound device (Mindray DC-N3, Shenzhen Mindray Bio-Medical Electronics Co. Ltd, China). All examinations were performed without sedation. Depending on the size of the patient, 5-11 MHz multifrequency probes were used. Two-dimensional (2D), M-mode (or motion mode) and pulse wave (PW) Doppler examination techniques were used in echocardiographic examination. The right 4-6 intercostal spaces were shaved. After the area was cleaned with alcohol, ultrasound gel was applied and waited for 1 minute for better conductivity. Then, right parasternal short axis (PSAX) and right parasternal long axis (PLAX) were taken and the heart was examined. M-mode measurements were made using the Teichholz method (Teicholz et al. 1976). To obtain left ventricular images, the cursor was positioned perpendicular to the interventricular septum (IVS) and left ventricular posterior wall (LVPW) at the level just posterior to the chordae tendineae. The following parameters were recorded: Left ventricular internal diameter in diastole (LVIDd) and systole (LVIDs), IVS thickness in diastole (IVSd) and IVS thickness in systole (IVSs), LVPW thickness in diastole (LVPWd) and LVPW thickness in systole (LVPWs). Directing the transducer slightly anteriorly and towards the spine allowed the aorta and left atrium to be visualized. The following measurements were recorded: left atrium (LA) diameter, aorta (Ao) diameter.

Ultrasonographic measurement of main pulmoner arter/aorta ratios

The diameter of the main pulmonary artery (MPA) (below the pulmonary valve) was measured at the level of the pulmonary valve in the right parasternal short axis view. The aortic (Ao) diameter was then measured.

The MPA diameter was divided by the Ao diameter (Figure 1).

Statistical analysis

Statistical analyses were performed with SPSS 25.0 (Chicago, IL, USA). Descriptive statistics were expressed as frequency and percentage. The conformity of the data to normal distribution was evaluated by Shapiro-Wilk test, histogram and Q-Q graphs. Data were expressed as median (25th and 75th percentile) or mean±standard deviation (SD). One-way ANOVA test (alternative, Kruskal Wallis) test was used for comparisons between groups. Tukey and Bonferroni tests were used for multiple comparisons. The relationship between pulmonary artery and body weight and age variables were evaluated using Pearson and Spearman rho correlation test. Statistical significance level was accepted as $P<0.05$.

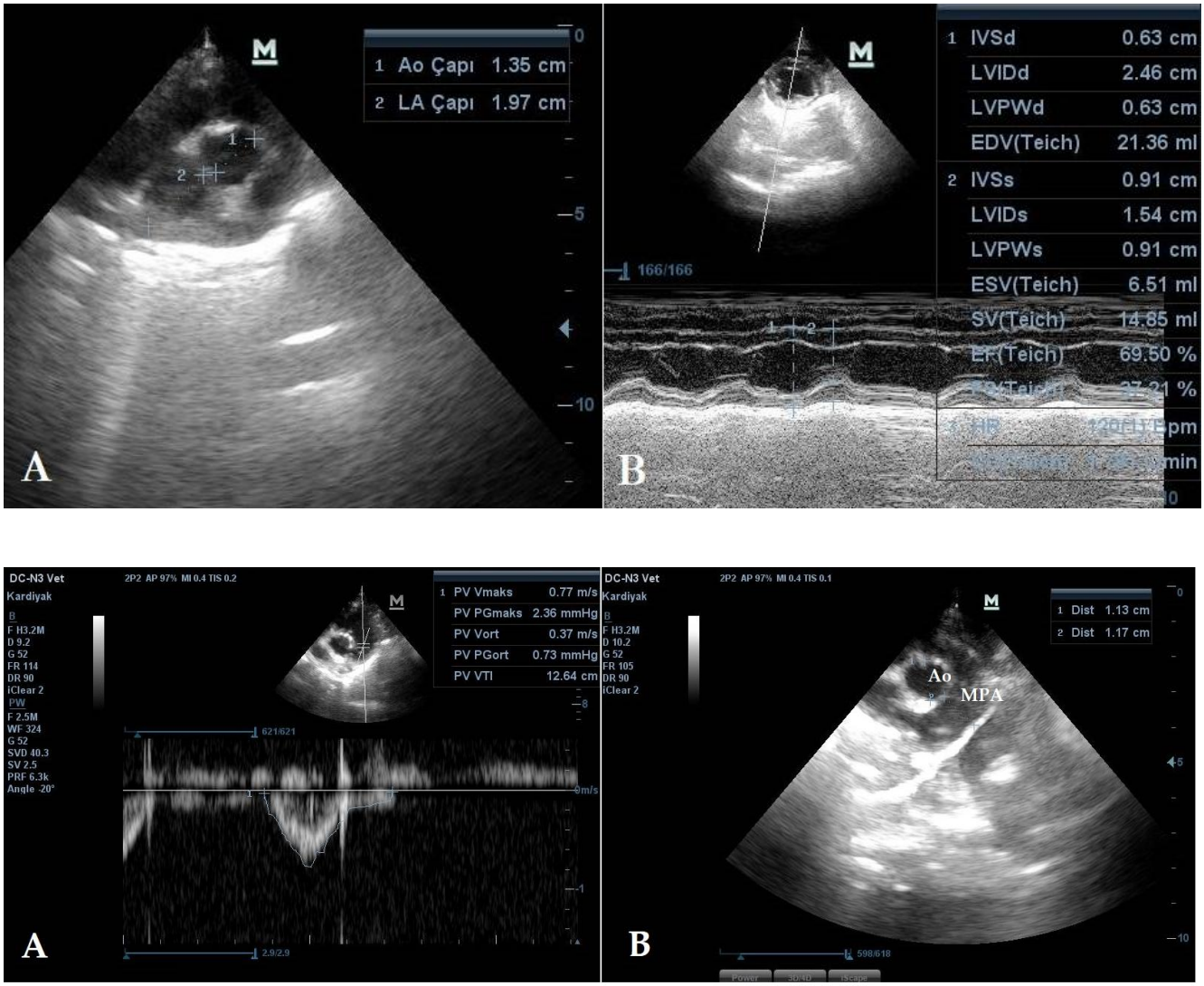

Figure 1. This sonogram was obtained from right parasternal short axis view. (**A**) Pulmonary valve PW Doppler and (**B**) pulmonary artery 2D echocardiograpic measurements **Ao**: Aorta; **MPA**: main pulmonary artery. **Vmaks**: Pulmonary valve maximum velocity, **PGmaks**: Maximum systolic pressure gradient, **Vort**: Mean velocity, **PGort**: Mean pressure gradient of pulmonic valve, **VTI**: Velocity time integral

RESULTS

Of the ninety dogs, 48 (53.3%) were female and 42 (46.7%) were male. Median body weight was 6.20 (4.80-9.80) kg. The most common dog breeds were Terrier (25.6%, 23/90), followed by Pomerian (18.9%, 17/90), Russian Poodle (13.3%, 12/90), crossbreed (7.8%, 7/90), Pekingese (6.7%, 6/90), Pug (6.7%, 6/90) and other dog breeds (number of animals less than 3).

Physical Examination Findings

Physical examination of dogs with tracheobronchitis revealed cough (100%, 90/90), tracheal tenderness (100%, 90/90), dyspnea (100%, 90/90), tachypnea (68.9%, 62/90), anorexia (60%, 54/90), and weakness

(40%, 36/90). When the degree of dehydration was examined, it was estimated that 73.3% were normal 66/90), 18.9% were mild (17/90), 4.4% were (moderate (4/90), and 2.2% were severely dehydrated (2/90). Enlargement of prescapular lymph nodes was detected in 70% (63/90) of dogs with tracheobronchitis. When the color of the mucous membranes was examined, it was determined that 66.7% were normal, 17.8% were pale, and 15.6% were hyperemic. It was determined that the mean/median pulse and respiratory rate of dogs with tracheobronchitis were higher than the reference values specified for dogs (Table 1) (Cugmas et al. 2020; MSD Veterinary Manual).

Complete Blood Count Findings

Complete blood count values of dogs with tracheobronchitis were within the reference values (Moritz et al. 2004; López and Mesa 2021) specified for dogs (Table 2).

Table 1. Physical examination findings of dogs with tracheobronchitis

Variables	Mean±SD	Median (25^{th}-75^{th} percentile)	Reference Range (Cugmas et al. 2020; MSD Veterinary Manual)
T (°C)	38.9±0.63	39.0 (38.6-39.3)	37.5-39.2
RR (min)	63.85±33.22	56 (40-72)	18.0-34.0
HR (bpm)	133.81±28.28	130 (120-150)	70.0-120.0
CRT (sec)	2.39±0.66	2.00 (2.00-3.00)	<3.0 sec

Data were expressed mean and standard deviation (SD) and median (25^{th}–75^{th} percentile). **CRT;** Capillary refill time, **HR;** heart rate, **T:** body temperature, **RT:** Respiration rate.

Table 2. Complete blood count results of dogs with tracheabronchitis

Variables	Mean±SD	Median (25^{th}-75^{th} percentile)	Reference Range (Moritz et al. 2004; López and Mesa 2021)
WBC (10^9/L)	12.87±5.44	11.20 (8.75-15.50)	6.00-17.00
Lymph (10^9/L)	2.36±0.93	2.30 (1.70-2.75)	0.90-5.00
Mono (10^9/L)	0.94±0.46	0.80 (0.60-1.20)	0.30-1.50
Neut (10^9/L)	9.48±5.05	8.20 (5.95-11.50)	3.50-12.00
Lymp (%)	20.40±8.36	20.10 (14.30-25.05)	9.00-47.00
Mono (%)	6.99±2.06	7.00 (5.45-7.95)	2.00-12.00
Neut (%)	72.55±9.15	73.30 (67.30-78.95)	42.00-84.00
Eos (%)#	0.05±0.01	0.00 (0.00-0.00)	1.00-18.00
RBC (10^{12}/L)	6.87±1.04	6.94 (6.14-7.46)	5.50-8.50
Hgb (g/dL)	15.94±2.51	16.00 (14.35-17.75)	12.00-18.00
Hct (%)	46.28±8.00	47.60 (40.35-52.10)	37.00-55.00
MCV (fL)	67.30±5.30	68.90 (65.40-70.75)	60.00-72.00
MCH (pg)	23.25±1.73	23.50 (21.90-24.50)	19.50-25.50
MCHC (g/dL)	34.41±2.30	34.10 (33.40-35.50)	32.00-38.50
RDWa (fL)	50.31±2.90	50.10 (48.30-52.35)	35.00-65.00
RDW (%)#	14.62±1.53	14.30 (13.55-15.60)	12.00-17.50
PLT (10^9/L)	321.08±119.56	296.00 (248.50-385.50)	200.00-500.00
MPV (fL)	6.61±0.90	6.50 (6.05-7.10)	5.50-10.50

Data were expressed mean and standard deviation (SD) and median (25^{th}–75^{th} percentile). **RBC**; Red Blood Cell, **Hct**; hematocrit, **Hgb**; hemoglobin concentration, **Lymph**; lymphocyte, **Neut**; Neutrophil, **Mono**; monocyte, **Eos**; Eosinophil, **MCV**; mean corpuscular volume, **MCH**; mean corpuscular hemoglobin volume, **MCHC**; mean corpuscular hemoglobin concentration, **WBC**; White Blood Cell, **PLT**; platelet, **RDW**; Red cell distribution, **RDWa**; absolute value of the width of the distribution of red blood cells, **MPV**; mean platelet volume.

Blood Pressure Findings

Mean SBP (148.74±21.34 mmHg), mean DBP (104.96±27.84 mmHg) and mean MAP (121.26±23.97 mmHg) values of the dogs with tracheobronchitis were slightly higher than the reference values specified for dogs (Acierno et al. 2018) (Table 3).

Echocardiographic Findings

The mean MPA diameter, Ao diameter and MPA/Ao ratio of dogs with tracheobronchitis were calculated as 1.20±0.31 cm, 1.37±0.36 cm and 0.90±0.16, respectively. PW Doppler measurements are presented in Table 4. There was a strong positive correlation between body weight and pulmonary artery diameter (r=0.754, P<0.001). The mean LA diameter, Ao diameter and LA/Ao ratio of dogs with tracheobronchitis were calculated as 1.90±0.56 cm, 1.37±0.46 cm and 1.42±0.25, respectively. M-mode and 2D echocardiographic measurements are presented in Table 5.

In the present study, no statistically significant difference was found between dog breeds in terms of MPA/Ao ratios. However, the mean Ao diameter of Pomeranian (1.03±0.14 cm) was lower than that of Terrier (1.30±0.26 cm), Russian Poodle (1.29±0.18 cm) and crossbreed (1.57±0.39 cm) (P=0.005, P=0.018, P=0.001, respectively). PW Doppler and 2D measurements according to dog breeds is presented in Table 6.

Table 3. Blood pressure values obtained from the right forelimbs of dogs with tracheobronchitis

Variables	Mean±SD	Median (25^{th}-75^{th} percentile)	**Reference Ranges** (Acierno et al. 2018)
SBP (mmHg)	148.74±21.34	144.00 (134.00-167.00)	90-140 mmHg
DBP (mmHg)	104.96±27.84	109.00 (83.00-119.00)	50-80 mmHg
MAP (mmHg)	121.26±23.97	122.00 (103-141.00)	60-100 mmHg

Data were expressed mean and standard deviation (SD) and median (25^{th}-75^{th} percentile). **SBP:** Systolic blood pressure, **DBP:** Diastolic blood pressure, **MAP**: Mean arterial pressure.

Table 4. Pulsed wave (PW) Doppler and two-dimensional (2D) echocardiographic measurements obtained from right parasternal short axis (PSAX) views in dogs with tracheobronchitis

Variables	Mean±SD	Median (25^{th}-75^{th} percentile)	Reference Ranges (Esser et al. 2016; Vurucu et al., 2021; Romito et. al., 2023)
MPA (cm)	1.20±0.31	1.12 (1.00-1.38)	0.8-1.5 cm
Ao (cm)	1.37±0.36	1.30 (1.13-1.53)	1.2-1.8 cm
MPA/Ao	0.90±0.16	0.88 (0.79-0.95)	≤1.0
Vmax (m/s)	0.88±0.20	0.83 (0.73-0.99)	0.6-1.0 m/s
PGmax (mmHg)	3.25±1.56	2.77 (2.12-3.90)	<20 mmHg
Vmean (m/s)	0.43±0.12	0.41 (0.35-0.48)	0.6-1.3 m/s
PGmean (mmHg)	1.05±0.63	0.89 (0.64-1.23)	<15 mmHg
VTI (cm)	15.71±8.54	14.53 (12.31-17.27)	8-15 cm

Data were expressed mean and standard deviation (**SD**) and median (25th–75th percentile). **Ao**: Aorta diameter, **MPA**: Main pulmonary artery, **MPA/Ao:** Main pulmonary artery/Aorta ratio, **Vmax**: Pulmonary valve maximum velocity, **PGmax**: Maximum systolic pressure gradient, **Vmean**: Mean velocity, **PGort**: Mean pressure gradient of pulmonic valve, **VTI**: Velocity time integral.

Table 5. M-mode and 2D echocardiographic measurements obtained from right parasternal long and short axis views in dogs with tracheobronchitis

Variables	Mean±SD	Median (25th-75th percentile)
LA (cm)	1.90±0.56	1.82 (1.51-2.11)
Ao (cm)	1.37±0.46	1.24 (1.07-1.53)
LA/Ao (No unit)	1.42±0.25	1.39 (1.28-1.52)
IVSd (cm)	0.75±0.21	0.74 (0.56-0.80)
LVIDd (cm)	2.62±0.67	2.40 (2.11-3.02)
LVPWd (cm)	0.70±0.20	0.69 (0.51-0.86)
EDV (mL)	27.77±18.38	20.14 (14.64-35.69)
IVSs (cm)	1.04±0.21	0.97 (0.86-1.26)
LVIDs (cm)	1.47±0.46	1.22 (1.09-1.83)
LVPWs (cm)	1.02±0.25	0.97 (0.89-1.09)
ESV (mL)	6.81±5.91	3.53 (2.57-10.11)
SV (mL)	20.96±12.95	16.61 (11.28-25.12)
EF (%)	77.25±7.24	78.97 (71.48-82.16)
SI (No unit)	40.36±12.13	41.15 (29.56-51.29)
FS (%)	43.95±6.68	43.15 (38.71-48.65)
CO (L/min)	2.91±1.73	2.40 (1.56-4.07)
CI (No unit)	5.33±1.92	5.37 (3.72-6.06)

Data were expressed mean and standard deviation (SD) and median (25th-75th percentile). **BSA**: Body surface area, **LA**: Left Atrium Diameter, **Ao**: Aorta Diameter, **LA/Ao**: Left atrium diameter/Aorta diameter, **IVSd**: Interventricular septal thickness at end diastole, **LVIDd**: Left ventricular internal diameter at end diastole, **LVPWd**: Left ventricular posterior wall thickness at end diastole, **EDV**: End-diastolic left ventricular volume, **IVSs**: Interventricular Septal thickness at end-systole, **LVIDs**: Left ventricular internal diameter at end systole, **LVPWs**: Left ventricular posterior wall thickness at end systole, **ESV**: End-systolic left ventricular volume, **SV**: Stroke volume, **EF**: Ejection fraction, **SI**: SV Index (SI = SV/BSA), **FS**: Fractional shortening [FS (No unit)= (LVIDd (cm)-LVIDs (cm)/LVIDd (cm)], **CO**: Cardiac output (CO= SV×HR), **CI**: CO index (CI= CO/BSA)

Table 6. Comparison of MPA diameter and pulmonary valve PW Doppler measurements between dog breeds

Variables	Terrier (n=23)	Pomeranian (n=17)	Russian Poodle (n=12)	Pekingese (n=6)	Pug (n=6)	Crossbreed (n=7)	P Values
LA (cm)	1.82±0.45	1.55±0.35	1.82±0.28	1.58±0.28	1.90±0.39	2.12±0.77	0.088
Ao (cm)	1.30±0.26^{b}	1.03±0.14^{a}	1.29±0.18^{b}	1.11±0.13^{ab}	1.23±0.19^{ab}	1.57±0.39^{b}	**<0.001**
LA/Ao	1.40±0.21	1.51±0.30	1.40±0.09	1.42±0.13	1.59±0.41	1.34±0.27	0.424
MPA (cm)	1.16±0.19	1.01±0.23	1.09±0.25	1.07±0.08	1.25±0.22	1.39±0.40	0.085
MPA/Ao	0.88±0.07	0.95±0.23	0.81±0.14	0.97±0.21	0.94±0.16	0.87±0.09	0.469
Vmax (m/s)	0.81±0.08^{ab}	0.88±0.25^{ab}	1.00±0.21^{a}	0.83±0.23^{ab}	0.69±0.20^{b}	0.68±0.16^{ab}	**0.024**
PGmax (mmHg)	2.68±0.53^{ab}	3.35±2.09^{ab}	4.17±1.76^{a}	2.93±1.66^{ab}	2.03±1.28^{b}	1.91±0.92^{ab}	**0.027**
Vmean (m/s)	0.40±0.07	0.46±0.17	0.47±0.10	0.39±0.08	0.37±0.12	0.35±0.07	0.091
PGmean (mmHg)	0.82±0.28	1.21±0.98	1.28±0.52	0.81±0.35	0.75±0.58	0.64±0.31	0.056
VTI (cm)	14.00±2.76	16.86±4.25	15.69±4.39	14.84±5.05	11.31±2.41	14.43±5.25	0.871

Data were expressed mean and standard deviation (SD) and median (25th–75th percentile). **LA**: Left Atrium Diameter, **Ao**: Aorta Diameter, **LA/Ao**: Left atrium diameter/Aorta diameter, **MPA**: Main pulmonary artery, **MPA/Ao:** Main pulmonary artery/Aorta ratio, **Vmax**: Pulmonary valve maximum velocity, **PGmax**: Maximum systolic pressure gradient, **Vmean**: Mean velocity, **PGort**: Mean pressure gradient of pulmonic valve, **VTI**: Velocity time integral. ^{a,b}Values within a row with different superscripts differ significantly at P<0.05. Other dog breeds were not included in the comparison due to their small numbers.

DISCUSSION

In this study, echocardiographic characteristics and MPA/Ao ratio were determined in dogs with tracheobronchitis, mostly small breed dogs brought to the clinic with respiratory difficulties. In our study, no abnormalities were observed in right and left ventricular functional indicators based on m-mode, 2D and PW Doppler measurements in dogs with tracheobronchitis. In the present study, the MPA/Ao ratio was found to be between the values reported for healthy dogs.

Tracheobronchitis is an inflammation of the trachea and bronchi (Rozanski et al. 2020). It typically affects the conducting airways, which are responsible for air transport, rather than directly influencing the blood vessels or the pulmonary vasculature (Rozanski et al. 2020). The MPA/Ao is presently the sole echocardiographic metric of pulmonary artery size recognized by the ACVIM recommendations for the noninvasive identification of pulmonary hypertension in canines (Reinero et al. 2020). In veterinary medicine, echocardiographic objective assessment of the MPA has been evaluated in dogs with healthy (Serres et al. 2007; Grosso et al. 2023) and pulmonary hypertension (PH) (Visser et al. 2016; Reinero et al. 2020). Echocardiography (Reinero et al. 2020), computed tomography (Truong et al. 2012) and cardiac magnetic resonance imaging (Burman et al. 2016) have linked PH severity to proximal MPA enlargement, stiffness, and impaired distensibility (Campbell 2007; Augustine et al. 2018; Reinero et al. 2020). In a study, the MPA/Ao ratio increased in proportion to the severity of pulmonary hypertension in dogs diagnosed with mild [1.05 (0.99-1.15)], moderate [1.1 (1.04-1.17)] and severe [1.24 (1.13-1.35)] PH compared to the control group [0.92 (0.89-0.98)] (Grosso et al., 2025). Serres et al. (2007) conducted an echocardiographic assessment of MPA in 45 healthy dogs utilizing 2D imaging from the right parasternal short axis. According to their findings, the MPA/Ao reference intervals ranged from 0.80 to 1.15 (Serres et al. 2007). In another study, MPA/Ao reference ranges of 22 healthy dogs were reported as 0.80 to 1.08 (Visser et al. 2016). Grosso et al. (2023) reported a range of 0.78 to 1.01 for 269 healthy dogs. In the present study, the MPA/Ao ratio of dogs with tracheobronchitis (0.90±0.16) was within the reference values specified for healthy dogs (Serres et al. 2007; Visser et al. 2016; Grosso et al., 2023; Grosso et al., 2025). In a study investigating echocardiographic characteristics in dogs with and without PH secondary to respiratory diseases, PA diameter and PA/Ao ratio were defined (Yuchi et al. 2023). PA/Ao ratio was calculated as 0.8 (0.8-0.9) in healthy dogs, 0.8 (0.8-0.8) in no or low PH probability, 1.0 (0.9-1.0) in without PH signs, 1.0 (1.0-1.0) in with PH signs (Yuchi et al. 2023). The MPA/Ao ratio of dogs with tracheobronchitis in our study (mean 0.90±0.16) was closer to dogs no or low PH probability (median 0.8 [0.8-0.8]) and healthy dogs (median 0.8 [0.8-0.9]) (Yuchi et al. 2023). The findings from this study indicate that tracheobronchitis does not cause vascular changes that could affect pulmonary pressure.

Gentile-Solomon et al. (2016) found that the MPA showed a positive linear relationship to body weight (R^2= 0.795). In another study, the MPA showed a positive linear relationship to body weight (R^2=0.859) (Grosso et al. 2023). In the present study, a strong positive correlation (r=0.754, P<0.001) was observed between the MPA and the body weight in dogs with tracheobronchitis.

In dogs with tracheobronchitis, basic laboratory tests, including a complete blood count, are useful in determining general health and would be expected to be largely normal in a dog with tracheobronchitis or chronic bronchitis (Kumrow and Rozanski 2012). In a study, no significant difference was reported between dogs with infectious tracheabronchitis and healthy dogs in terms of WBC and granulocyte counts (Koçhan et al. 2017). In the present study, WBC and differentials (lymph, neut, etc..) in dogs with tracheobronchitis were found to be within the reference ranges (Moritz et al. 2004; López and Mesa 2021) for healthy dogs. A possible explanation for our findings may be related to the fact that most cases of tracheobronchitis are localized, chronic and with a mild infection. Tracheal bronchitis usually involves localized inflammation of the upper respiratory tract without systemic involvement. Because the infection is confined to the trachea and bronchi, it does not always trigger a systemic immune response to alter leukocyte levels (Rozanski et al. 2020). In chronic or long-term cases, the immune system can adapt to ongoing low-level inflammation and prevent significant changes in leukocyte levels. Most cases of tracheal bronchitis result from mild infections (especially those caused by canine parainfluenza or *Bordetella bronchiseptica*). Mild infections usually do not trigger an immune response strong enough to increase WBC counts (Rozanski et al. 2020). In addition, in immunocompetent dogs, the immune system can effectively control the infection locally without the need to mobilize large numbers of circulating white blood cells (Kumrow and Rozanski 2012; Reagan and Sykes 2020).

Blood pressure (BP) is an important parameter to measure to assess cardiovascular system function (Sierra and Savino 2015ab). Measuring blood pressure in dogs (Ekinci et al. 2024), cats (Güneş et al. 2021) and calves (Deniz et al. 2022) using the oscillometric method is a practical method. In the present study, mean blood pressure values (SBP, DBP, MAP) of dogs with tracheobronchitis were found to be slightly higher than the reference values reported by Acierno et al. (2018) for healthy dogs. Stress and anxiety in the clinic setting, combined with stress from handling to take a blood pressure measurement, can result in increased readings and a false diagnosis of hypertension (Sierra and Savino 2015ab).

In the present study, the mean pulse frequency and respiratory rate obtained from dogs with tracheabronchitis were higher than the values reported for healthy dogs (Cugmas et al. 2020; MSD Veterinary Manual). Other vital parameters (body temperature, capillary refilling time) were within the normal range. Body temperature and white blood cell counts usually remain normal in dogs with tracheobronchitis (MSD Veterinary Manual).

In the present study, the mean maximum velocity of blood flow (Vmax) and maximum pressure gradient (PGmax) values of Pugs were lower than those of Russian poodles. Pugs suffer from Brachycephalic Obstructive Airway Syndrome, which often leads to upper airway obstruction (Wiegel et al. 2022). There are also slight differences in heart size and positioning within the chest cavity in Pugs (Romito et al. 2023). The altered geometry of the heart valves (especially the tricuspid and pulmonary valves) can lead to breed-specific variations in flow velocity and pressure gradients. Due to the Pug's compact chest and short neck, echocardiographic angles may be suboptimal, which may affect the accuracy of measured Vmax and PG max values (Kavitha et al. 2020; Wiegel et al. 2022). These factors may explain the difference in echocardiographic measurements obtained from Pugs in the current study.

In the present study, no statistically significant difference was found between dog breeds in terms of PA and MPA/Ao ratios. However, the mean Ao dia (cm) of Pomeranian dog breed was lower than that of Terrier and Russian Poodle dogs. Reference values from breed-specific echocardiographic studies have been found to differ significantly from the overall population of healthy dog breeds (Jacobson et al. 2013; Vurucu et al. 2021). As a result, breed-specific echocardiographic reference ranges may be more useful in preventing misunderstanding of echocardiographic findings (Kayar et al. 2006). The difference in mean Ao diameters among the dog breeds included in the study can be explained by differences in body size and somatotype (Jacobs et al. 1988; Morrison et al. 1992).

The main limitation of the present study is that echocardiographic measurements obtained from the dog breeds included in the study could not be compared with healthy control groups. Another limitation is that nasoparigeal swabs, transtracheal and bronchoalveolar lavage fluids could not be obtained from the dogs included in the study. Therefore, common pathogens causing respiratory tract infections in dogs (canine parainfluenza, Bordetella bronchiseptica, etc.) could not be identified. The diagnosis of tracheabronchitis was based only on anamnesis, physical examination findings, diagnostic imaging (thoracic radiography) and laboratory (complete blood count) findings. Furthermore, the study lacked assessment of intra- and inter-observer variability.

CONCLUSION

In conclusion, the MPA/Ao ratio in dogs with tracheobronchitis included in the study was between the values reported for healthy dogs. Based on basic echocardiographic findings and pulmonary artery measurements, no signs of pulmonary hypertension were detected in dogs with tracheobronchitis. However, it can be argued that due to the progressive nature of PH and its poor prognosis, a more comprehensive echocardiographic evaluation may be useful to identify dogs with the potential to develop PH.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: GE contributed to the project idea, design and execution of the study. CB and GE contributed to the acquisition of data. CB and GE analysed the data. CB and GE drafted and wrote the manuscript. CB and GE reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Ethical approval: "This study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k1). The data, information and documents presented in this article were obtained within the framework of academic and ethical rules."

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RESEARCH ARTICLE

Isolation and Antimicrobial Susceptibility of Selected Bacterial Pathogens from Pneumonic Lung Samples of Sheep and Goats

Muazzez YEŞİLYURT[1*], Özgül GÜLAYDIN[1]

[1]Department of Microbiology, Faculty of Veterinary Medicine, Siirt University, Siirt, Türkiye

ABSTRACT

Pneumonia in sheep and goats causes significant economic losses worldwide. This study aimed to isolate and identify *Pasteurella multocida* (*P. multocida*), *Mannheimia haemolytica* (*M. haemolytica*), *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Histophilus somni* (*H. somni*) and *Trueperella pyogenes* (*T. pyogenes*), as well as to determine their antimicrobial susceptibilities in lung samples from sheep and goats that were slaughtered at the Siirt Municipal Slaughterhouse, where pneumonic lesions were detected post-mortem. In the study, 429 lung samples were collected from 270 goats and 159 sheep slaughtered in the Siirt Municipal Slaughterhouse. As a result of the PCR analysis, *P. multocida* was identified in 8 (1.86%) out of the lung samples, *M. haemolytica* in 8 (1.86%), *E. coli* in 10 (2.33%), *T. pyogenes* in 2 (0.46%), and *Klebsiella* spp. in 1 (0.23%), while *H. somni* was not identified. The antimicrobial susceptibilities of the obtained isolates were determined by the Kirby-Bauer disc diffusion method. According to antimicrobial susceptibility test results, it was determined that all *P. multocida* and *M. haemolytica* isolates were sensitive to cefpodoxime, ceftiofur, enrofloxacin, florfenicol, and spectinomycin; all *T. pyogenes* isolates were determined to be susceptible to danofloxacin, amoxicillin, clavulanic acid, oxytetracycline, erythromycin, ceftiofur, florfenicol, sulfamethoxazole/trimethoprim; and all *E. coli* isolates were susceptible to ertapenem, piperacillin/tazobactam, and cefoxitin. Consequently, it was determined that especially *P. multocida, M. haemolytica* and *E. coli* strains may cause pneumonia cases in sheep and goats raised in the Siirt region. Furthermore, the isolated strains were generally susceptible to antibiotics as a result of antimicrobial susceptibility tests.

Keywords: Antimicrobial susceptibility, Goat, Pneumonia, Sheep.

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Koyun ve Keçilerin Pnömonik Akciğer Örneklerinden Bazı Bakteriyel Patojenlerin İzolasyonu ve Antimikrobiyal Duyarlılığı

ÖZ

Koyun ve keçilerde meydana gelen pnömoni dünya çapında önemli ekonomik kayıplara neden olur. Bu çalışmada, Siirt Belediye Mezbahasında kesilen ve post-mortem pnömonik lezyonlar tespit edilen koyun ve keçilerin akciğer örneklerinde *Pasteurella multocida* (*P. multocida*), *Mannheimia haemolytica* (*M. haemolytica*), *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Histophilus somni* (*H. somni*) ve *Trueperella pyogenes*'in (*T. pyogenes*) izolasyon ve identifikasyonunun yapılması ve antimikrobiyal duyarlılıklarının belirlenmesi amaçlanmıştır. Çalışmada, Siirt Belediye Mezbahasında kesilen 270 keçi ve 159 koyuna ait 429 akciğer örneği toplandı. Yapılan PCR analizi sonucunda, alınan akciğer örneklerinin 8 (%1.86)'inden *P. multocida*, 8 (%1.86)'inden *M. haemolytica*, 10 (%2.33)'undan *E. coli*, 2 (%0.46)'sinden *T. pyogenes*, 1 (% 0.23)'inde de *Klebsiella* spp. izole edilirken, örneklerde *H. somni* belirlenmedi. Elde dilen izolatların antimikrobiyal duyarlılıkları Kirby-Bauer disk difüzyon yöntemi ile belirlendi. Antimikrobiyal duyarlılık test sonuçlarına göre; *P. multocida* ve *M. haemolytica* izolatlarının tamamının sefpodoksim, seftiofur, enrofloksasin, florfenicol ve spektinomisine, *T. pyogenes* izolatlarının tamamının danofloksasin, amoksisilin klavulanik asit, oksitetrasiklin, eritromisin, seftiofur, florfenikol, sulfametoksazol/trimetoprime; *E. coli* izolatlarının tamamının ise; ertapenem, piperasilin/tazobaktam ve sefoksitine duyarlı olduğu belirlendi. Sonuç olarak bu çalışmada Siirt bölgesinde yetiştiriciliği yapılan koyun ve keçilerde meydana gelen pnömoni olgularına özellikle *P. multocida*, *M. haemolytica* ve *E. coli* suşlarının neden olabileceği belirlendi. Ayrıca izole edilen suşların yapılan antimikrobiyal duyarlılık testleri sonucunda antibiyotiklere genel olarak duyarlı olduğu sonucuna varıldı.

Anahtar Kelimeler: Antimikrobiyal duyarlılık, Keçi, Koyun, Pnömoni.

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ORCID ID; *MY: 0000-0003-4195-6335, ÖG: 0000-0001-8376-2008*

*Corresponding author e-mail: muazzez.yesilyurt@siirt.edu.tr

INTRODUCTION

Small ruminant breeding, which is adapted to the geographical conditions of Türkiye and can make use of pastures throughout the year and allows the utilisation of inarable lands, takes place among important economic activities (Bakır and Mikail 2019). However, small ruminant breeding has been negatively affected and suffers major economic losses due to respiratory diseases that are effective on a herd basis (Abera and Mossie 2023). Although the prevalence of respiratory diseases in small ruminants is around 5.6%, 50% of the herd mortality is caused by respiratory tract infections (Chakraborty et al. 2014; Rahman et al. 2022).

Respiratory diseases in sheep and goats are caused by immunosuppression, bacterial and viral infections, parasitic infestations, environmental factors, and stress (Mahmoud et al. 2005; Chakraborty et al. 2014; Valadan et al. 2014; Nejiban and Al-Amery 2018; Abera and Mossie 2023). *Pasteurella multocida* (*P. multocida*), *Mannheimia haemolytica* (*M. haemolytica*), *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Histophilus somni* (*H. somni*), *Bibersteinia trehalosi* (*B. trehalosi*), *Staphylococcus aureus* (*S. aureus*), *Proteus vulgaris* (*P. vulgaris*) and *Trueperella pyogenes* (*T. pyogenes*) are frequently isolated and identified in pneumonia cases among small ruminants (Wang et al. 2012; Marru et al. 2013; Hanthorn et al. 2014; Headley et al. 2018; Babu et al. 2019; Mahrous et al. 2023).

M. haemolytica, which causes significant acute pneumonia outbreaks in newborn and weaned lambs, has become one of the important pathogens of the respiratory system, causing serious respiratory diseases in ruminants (Fernandez et al. 2016). In addition, *M. haemolytica* causes *P. multocida* to better colonise in the lungs and cause serious disease (Dabo et al. 2007). *E. coli* and *K. pneumoniae* are among the pathogens isolated from pneumonia cases with high mortality and morbidity rates (Wang et al. 2012). Particularly, *E. coli* and *K. pneumoniae* isolates producing extended-spectrum β-lactamase (ESBL) are of great importance because they cause the development of multiple resistance (Krishnamurthy et al. 2013). The global spread of ESBLs creates public health concerns in both developed and developing countries in terms of the development of multiple drug resistance (Chong et al. 2011; Alali et al. 2021).

National and international studies have reported that *P. multocida*, *M. haemolytica*, *E. coli, K. pneumoniae, H. somni* and *T. pyogenes* strains, which play an important role in respiratory diseases, have been isolated from lung samples of sheep and goats, and their antimicrobial susceptibilities have been determined by the disc diffusion method. Related studies have reported that the isolation rate of *P. multocida* from sheep and goats varies between 0.7% and 20% (Özbey and Muz 2004; Tel and Keskin 2010; Jarikre et al. 2018; Singh et al. 2019). Similarly, the isolation rate of *M. haemolytica* ranges between 2% and 40%, *E. coli* between 2% and 15.38%, *K. pneumoniae* between 0.7 and 3%, *T. pyogenes* between 0 and 20%, and *H. somni* between 2% and 8.7% (Özbey and Muz 2004; Tel and Keskin 2010; Tesfaye et al. 2013; Gulaydın and Gurtürk 2018; El-Mashad et al. 2019; Thakur et al. 2019).

The number of ovine animals in Türkiye has been reported to be 52.363.410, while the number of ovine animals in the Siirt region is 1.197.307 (TÜİK 2023). Siirt has great importance for livestock activities due to its topographical structure and geography. Small ruminant breeding in the region provides an important source of livelihood for the people of the Siirt region, especially due to the production of meat, milk, leather, and fleece (Semerci and Çelik 2016; Bakır and Mikail 2019). Finding solutions to the problems in small ruminant breeding, an important source of livelihood for the people of the region would make significant contributions to the economy of both the region and the country. From this perspective, it is important to identify the aetiology of respiratory diseases prevalent among sheep and goats that are raised in the region and to evaluate effective treatment options. It was found that there had been no previous study on the subject in the region. Therefore, this study aimed to isolate and identify *P. multocida*, *M. haemolytica*, *K. pneumoniae*, *E. coli*, *H. somni* and *T. pyogenes* the causative bacteria of pneumonia in sheep and goats from lung samples and to determine their susceptibility to various antimicrobial agents.

MATERIALS and METHODS

Materials

The study material consisted of 429 lung samples collected from 270 goats and 159 sheep that were slaughtered in Siirt municipal slaughterhouse and whose lungs showed pneumonic lesions on macroscopic examination. Palm-sized lung samples were taken and were placed in phosphate buffer saline (PBS) containing 20% glycerine and stored at -20 °C until they were used.

Methods

Collection of lung samples

Lung samples were collected from sheep and goats slaughtered at the Siirt municipal slaughterhouse in Siirt province. As a result of the examinations, palm-sized sections were taken from the lungs where pneumonic lesions were observed macroscopically and samples were collected. The samples were transferred to sample collection containers with PBS containing 20% glycerine and delivered under cold chain conditions to the Department of Microbiology Laboratory of the Faculty of Veterinary Medicine at Siirt University.

Isolation and Identification of Bacterial Agents

The surface of the lung samples was cauterised and cut with a sterile scalpel, and a swab sample was taken from the inner part of the organ and transferred to the

Brain Heart Infusion Broth (Himedia, M210, Mumbai, India). Their medium was incubated at 37 °C for 24 hours (Besser et al. 2012; El-Mashad et al. 2019). At the end of the incubation, samples were inoculated onto MacConkey agar (Himedia, MH081, Mumbai, India), Eosin Methylene Blue (EMB) agar (Oxoid, CM0069, Hampshire, England) and the blood agar (Oxoid, CM0271, Hampshire, England), containing 5% sheep blood and incubated at 37 °C for 24-48 hours in an aerobic environment (Quinn et al. 2011). Also, they were inoculated on Columbia agar (Oxoid, CM0331, Hampshire, England) containing 5% sheep blood for isolation of *H. somni* and *T. pyogenes* and incubated at 37 ℃ in an atmosphere of 5-10% CO_2 for 24-48 hours (Humphrey and Stephens 1983; Ward et al. 2006). The colonies were Gram-stained. Catalase, oxidase, and various biochemical properties of the isolates were determined (Quinn et al. 2011).

Identification by Polymerase Chain Reaction

Suspected isolates were confirmed by PCR using species-specific primers. Table 1 shows the information about the specific primers to be used for this purpose.

DNA Extraction:

Genomic DNA was extracted using a commercial DNA isolation kit (GeneAll, ExgeneTM Clinic SV Mini, 108.101, Seoul, Korea).

PCR Amplification:

Commercial mastermix (2X PCR Mastermix, BioLabs, 1333-HY-100, Van, Turkiye,) was used to prepare the PCR mixture. For optimisation of the mixture, 5 µl of genomic DNA and 1.5 µl from each of the primers 10 (µM) were added to 12.5 µl of mastermix, and the total volume was completed up to 25 µl with PCR water. During the amplification process, the binding temperature was optimised according to the recommendations of the company where the primers were synthesised (Table 1). The optimization process included an, initial denaturation step at 94 °C for 10 min and a final extension step at 72 °C for 10 min. Amplification procedures are given in Table 1. Amplicons generated PCR were electrophoresed in agarose gel and analysed in a gel imaging system (Gen-Box ImagER, Ankara, Türkiye). *P. multocida* subsp. *multocida* ATCC® 43137, *M. haemolytica* ATCC®33396, *T. pyogenes* ATCC® 19411, *E. coli* ATCC® 25922, and *K. pneumoniae* strain identified by MALDI-TOF found in the culture collection in the microbiology laboratory were used as positive controls in the PCR analysis, and DNA-free PCR water was used as a negative control

Table 1 Primers and sequences used in the identification of isolates for PCR.

Species	Target Gene	Oligonukleotid (5'-3')	Amplification Protocol	Amplicon size (bp)	References
P. multocida	KMT1	F: ATCCGCTATTTACCCAGTGG R: GCTGTAAACGAACTCGCCAC	94 ℃ 60 s 58 ℃ 60 s 72 ℃ 60 s (35 cycles)	460	Townsend et al. 1998
M. haemolytica	PHSSA	F: TTCACATCTTCATCCTC R: TTTTCATCCTCTTCGTC	94 ℃ 60 s 48 ℃ 60 s 72 ℃ 60 s (35 cycles)	325	Hawari et al. 2008
E. coli	16S rRNA	F: CCCCCTGGACGAAGACTGAC R: ACCGCTGGCAACAAAGGATA	94 ℃ 60 s 55 ℃ 60 s 72 ℃ 60 s (35 cycles)	401	Wang XiaoRong et al. 2012

Klebsiella spp.	*glyA*	F: CGCGTACTATACGCCATGAACGTA R: ACCGTTGATCACTTCGGTCAGG	94 ℃ 60 s 62 ℃ 60 s 72 ℃ 60 s (35 cycles)	441	Sikrodia et al. 2022
K. pneumoniae	16S rRNA	F: ATTTGAAGAGGTTGCAAACGAT R: TTCACTCTGAAGTTTTCTTGTGTTC	94 ℃ 60 s 56 ℃ 60 s 72 ℃ 60 s (35 cycles)	130	Turton et al. 2010
H. somni	16S rDNA	F: GAAGGCGATTAGTTTAAGAG R: TTCGGGCACCAAGTATTCA	94 ℃ 60 s 55 ℃ 60 s 72 ℃ 60 s (35 cycles)	407	Angen et al. 2003
T. pyogenes	16S-23S rDNA	F:GTTTTGCTTGTGATCGTGGTGGTT ATGA R: AAGCAGGCCCACGCGCAGG	94℃ 60 s 63 ℃ 60 s 72 ℃ 60 s (35 cycles)	122	Ülbegi-Mohyla et al. 2010

Note: *P. multocida*: *Pasteurella multocida*; *M. haemolytica*: *Mannheimia haemolytica*; *E. coli*: *Escherichia coli*; *K. pneumoniae*: *Klebsiella pneumoniae*; *H. somni*: *Histophilus somni*; *Trueperella pyogenes*: *T. pyogenes*.

Determination of Antimicrobial Susceptibility

The susceptibility of the isolates to various antimicrobial agents was determined by the disc diffusion method.

Accordingly, ceftiofur (30 μg, Bioanalyse, Ankara, Türkiye), enrofloxacin (5 μg, Bioanalyse, Ankara, Türkiye), spectinomycin (100 μg, Liofilmchem, Roseto degli Abruzzi, Italy), cefpodoxime (10 μg, Liofilmchem, Roseto degli Abruzzi, Italy), tulathromycin (30 μg, Bioanalyse, Ankara, Türkiye), and florfenicol (30 μg, Bioanalyse, Ankara, Türkiye) were used for *P. multocida* and *M. haemolytica*.

Cefpodoxime (10 μg, Liofilmchem, Roseto degli Abruzzi, Italy), ceftazidime (30 μg, Bioanalyse, Ankara, Türkiye), aztreonam (30 μg, Bioanalyse, Ankara, Türkiye), cefotaxime (30 μg, Hımedia, Mumbai, India), ceftriaxone (30 μg, Oxoid, Hampshire, UK), cefoxitin (30 μg, Bioanalyse, Ankara, Türkiye), enrofloxacin (5 μg, Bioanalyse, Ankara, Türkiye), gentamicin (10 μg, Bioanalyse, Ankara, Türkiye), piperacillin-tazobactam (100/10 μg, Bioanalyse, Ankara, Türkiye), chloramphenicol (30 μg, Bioanalyse, Ankara, Türkiye), doxycycline (30 μg, Liofilmchem, Roseto degli Abruzzi, Italy), imipenem (10 μg, Liofilmchem, Roseto degli Abruzzi, Italy), streptomycin (10 μg, Bioanalyse, Ankara, Türkiye), trimethoprim-sulfamethoxazole (1.25/23.7 μg, Bioanalyse, Ankara, Türkiye) antibiotic discs were used for *E. coli* and *K. pneumoniae.*

Danofloxacin (5 μg, Bioanalyse, Ankara, Türkiye), amoxicillin-clavulanic acid (20/10 μg, Bioanalyse, Ankara, Türkiye), oxytetracycline (30 μg, Oxoid, Hampshire, UK), erythromycin (15 μg, Liofilmchem, Roseto degli Abruzzi, Italy), Ceftiofur (30 μg, Bioanalyse, Ankara, Türkiye), florfenicol (30 μg, Bioanalyse, Ankara, Türkiye), and trimethoprim-sulfamethoxazole (1.25/23.7 μg, Bioanalyse, Ankara, Türkiye) antibiotic discs were used for *T. pyogenes*.

At the end of the incubation period, the zone diameters that were formed around the antibiotic discs were measured. The measured values were compared with the criteria established by CLSI (2002; 2003; 2018; 2023) and EUCAST (2023), and the susceptibility, intermediate susceptibility, or resistance of the pathogens to antibiotics was determined.

RESULTS

Isolation and Identification

In the study, a total of 429 lung samples (159 sheep and 270 goats) with macroscopic findings of pneumonia were collected. As a result of bacteriological examination of the lung samples collected from sheep using conventional methods, 13 (8.17%) isolates were suspected of *P. multocida*, 10 (5.03%) isolates suspected of *M. haemolytica*, 10 (6.28%) isolates suspected of *E. coli*, 2 (1.25%) isolates

suspected of *Klebsiella* spp., 2 (1.25%) isolates were suspected of *H. somni*, and 5 (3.14%) isolates were suspected of *T. pyogenes* (Table 2). As a result of the PCR analysis, it was found that among 159 sheep lung samples, *P. multocida* was identified in 7 (4.40%) isolates, *M. haemolytica* in 7 (4.40%) isolates, *E. coli* in 6 (3.77%) isolates, *Klebsiella* spp. in 1 (0.62%) isolate, and *T. pyogenes* in 1 (0.62%) isolate. Bacteriological examination of 270 lung samples collected from goats by conventional methods indicated 5 (1.85%) isolates suspected of *P. multocida*, 3 (1.11%) isolates suspected of *M. haemolytica*, 4 (1.48%) isolates suspected of *E. coli*, 3 (1.11%) isolates suspected of *H. somni*, and 14 (5.18%) isolates suspected of *T. pyogenes* (Table 2).

Table 2: Distribution of isolates obtained from sheep and goat lung samples (n=429)

Agent	Sheep (%)	Goat (%)	Total (%)
P. multocida	4.40	0.37	1.86
M. haemolytica	4.40	0.37	1.86
E. coli	3.77	1.5	2.33
Klebsiella spp.	0.62	0	0.23
T. pyogenes	0.62	0.37	0.46
H. somni	0	0	0

Note: *P. multocida: Pasteurella multocida; M. haemolytica: Mannheimia haemolytica; E. coli: Escherichia coli; H. somni: Histophilus somni; Trueperella pyogenes: T. pyogenes.*

PCR analysis showed that 1 (0.37%) isolate tested positive for *P. multocida*, 1 (0.37%) isolate tested positive for *M. haemolytica*, 4 (1.5%) isolates tested positive for *E. coli*, and 1 (0.37%) isolate tested positive for *T. pyogenes* (Fig. 1). However, none of the isolates from both sheep and goat lung samples were not tested positive for *H. somni*. *K. pneumonia* was not isolated in goats by conventional and molecular methods.

Antimicrobial Susceptibility

All *P. multocida* and *M. haemolytica* isolates (100%) were susceptible to cefpodoxime, ceftiofur, enrofloxacin, florfenicol, and spectinomycin, while 25% of *P. multocida* isolates were resistant to tulathromycin and 25% of *M. haemolytica* isolates were moderately susceptible to tulathromycin.

All *T. pyogenes* isolates that were identified in the study were susceptible to danofloxacin, amoxicillin/clavulanic acid, oxytetracycline, erythromycin, ceftiofur, florfenicol, and sulfamethoxazole+trimethoprim.

All *E. coli* isolates (100%) were susceptible to cefoxitin, piperacillin/tazobactam, and ertapenem, while 10% of the isolates were resistant to cefpodoxime, aztreonam, gentamicin, enrofloxacin, sulfamethoxazole+trimethoprim, chloramphenicol, and ciprofloxacin.

On the other hand, *Klebsiella* spp. strain isolated from the lung samples were susceptible to cefoxitin, piperacillin/tazobactam, and ertapenem, and resistant to chloramphenicol and sulfamethoxazole+trimethoprim.

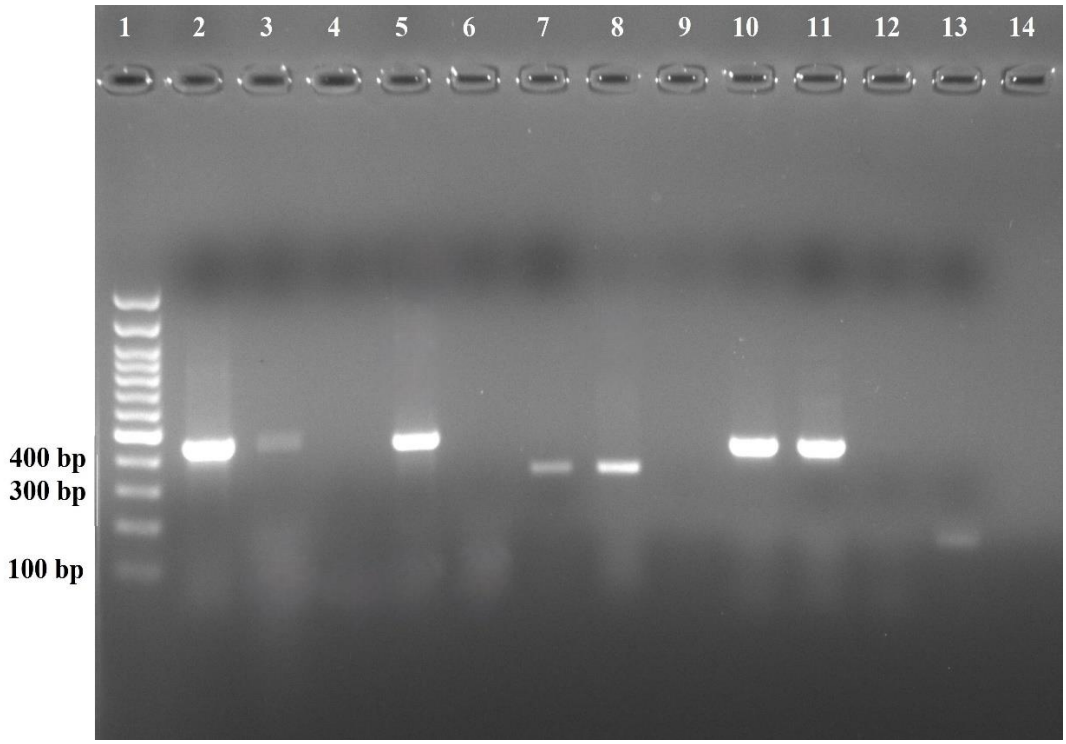

Figure 1: Agarose gel image of amplicons obtained as a result of PCR [1: 100 bp DNA marker; 2-3: *P. multocida* positive isolate (460 bp); 4: *P. multocida* negative control; 5: *Klebsiella* spp. positive isolate (441 bp); 6: *Klebsiella* spp. negative control; 7-8: *M. haemolytica* positive isolate (325 bp); 9: *M. haemolytica* negative control; 10-11: *E. coli* positive isolate (401 bp); 12: *E. coli* negative control; 13: *T. pyogenes* positive isolate (122 bp), 14: *T. pyogenes* negative control].

DISCUSSION

Respiratory tract infections in ovines have a multifactorial aetiology. To establish effective prevention-control strategies and treatment options against the disease, it is important to understand the aetiology (Asaye et al. 2015; Baykan et al. 2023). Antimicrobial treatment is inevitably essential for the effective treatment of bacterial pneumonia cases. However, the resistance in bacteria constrains the treatment options. It is necessary to monitor the resistance rates that develop in bacteria in order to achieve effective treatment of infectious diseases (Kılıç 2004; Gulaydin et al. 2021). This study aimed to identify *P. multocida*, *M. haemolytica*, *E. coli*, *K. pneumoniae*, *T. pyogenes* and *H. somni* isolates that cause respiratory tract infections in sheep and goats by PCR method as well as bacteriological methods and to determine the antimicrobial susceptibility results of the isolates.

A number of national studies have been conducted on the subject. In a prevalence study conducted by Ülgen et al. (1997) in Bursa, *K. pneumoniae* was isolated in 12.85% of 71 lamb lung samples, *E. coli* in 11.43%, *M. haemolytica* in 10% and *Histophilus* spp. in 5.71%. In a study conducted in Elazığ, *P. multocida* was detected in 15 (4.28%) of 350 sheep lung samples, *M. haemolytica* in 8 (2.3%) and *E. coli* in 10 (2.8%) (Özbey and Muz, 2004). A study conducted by Oruc (2006) in Konya reported that *M. haemolytica* was identified in 56.14% of 262 lung samples with pneumonia, *E. coli* was identified in 24.56%, and *P. multocida* was identified in 10.52%. A study conducted by Tel and Keskin (2010) in Şanlıurfa reported that they isolated and identified *M. haemolytica* at 12.5% and *P. multocida* at 31.6% from lung samples of sheep, and another study conducted by Özavcı et al. (2022) in the Aegean region reported that they isolated and identified *M. haemolytica* at 10% and *P. multocida* at 11% from lung samples of sheep and lamb with pneumoniae. Another study conducted in the Marmara region indicated that they identified *M. haemolytica* in 35.37%, *Klebsiella* spp. in 6.10%, and *P. multocida* in 8.54% of lung samples from sheep with pneumonia (Baykan et al. 2023). A study conducted by Tesfaye et al. (2013) in Ethiopia reported that they isolated and identified *P. multocida* in 2.4%, *M. haemolytica* in 25.4%, *H. somni* in 8.7%, and *Klebsiella* spp. in 1.6% of 960 nasal swab samples collected from sheep. In their study conducted in Egypt, El-Mashad et al. (2019) found *E. coli* in 15.38% and *K. pneumoniae* in 3.84% of 134 sheep lung samples. Likewise, a study conducted in India reported that they isolated and identified *M. haemolytica* in 18.75% and *P. multocida* in 14.58% of 96 sheep lung samples using bacteriological and molecular methods (Singh et al. 2019). Akane et al. (2022) reported that they identified *M. haemolytica* in 32.62% of 141 nasal swab samples.

When the results obtained from sheep lung samples were compared with the results of other studies, the rate of obtaining *E. coli* was found to be low compared to the results of the studies of Ülgen et al. (1997), Oruc et al. (2006) and El-Mashad et al. (2019). It was found to be higher according to the results of Özbey and Muz (2004). The rate of *M. haemolytica* presence was evaluated as low according to the results of Ülgen et al. (1997), Oruc (2006), Tel and Keskin (2010), Tesfaye et al. (2013), Singh et al. (2019), Akane et al. (2022) and Baykan et al. (2023), and higher according to the study results of Özbey and Muz (2004). In this study, the acquisition rate of *P. multocida* was evaluated as low compared to the results of Oruc (2006), Tel and Keskin (2010), Singh et al. (2019) and Baykan et al. (2019). Similarly *Klebsiella* spp. detection rate was found to be low compared to the study results of El-Mashad et al. (2019), Baykan et al. (2023) to the results of Tesfaye et al. (2013).

Studies have also been carried out to determine the aetiology of respiratory diseases in goats. In their study conducted in Elazığ, Özbey and Muz (2004) reported that they isolated and identified *P. multocida* in 0.7%, *M. haemolytica* in 4%, *E. coli* in 2.7%, and *Klebsiella* spp. in 0.7% of 150 goat lung samples. A study conducted by Baykan et al. (2023) in the Marmara region in 2023 reported that they examined a total of 25 goat lung samples and identified *P. multocida* in 8%, *M. haemolytica* in 20%, and *Klebsiella* spp. in 8% of the samples. Similarly, a study conducted by Ferdausi et al. (2008) in Bangladesh reported that they isolated *Pasteurella* spp. and *E. coli* in 11.7% and 6.7% of goat lung samples, respectively. Another study conducted in Mexico reported that *H. somni* was identified in 2.38% of 42 goat nasal swab samples (Pérez-Romero et al. 2011). Rashid et al. (2013) reported that *E. coli* was detected in 25% and *Pasteurella* spp. in 15% of goat lung samples in which gross abnormalities were detected, while Jarikre et al. (2018) detected *E. coli* in 13%, *P. multocida* in 20% and *M. haemolytica* in 40% of 150 goat lung samples. A study conducted by El-Mashad et al. (2019) in Egypt stated that they isolated *E. coli* in 7.69% of lung samples. Another study conducted by Adam et al. (2023) in Nigeria reported that they isolated and identified *Klebsiella* spp. in 51.72% of 58 goat lung samples and *E. coli* in 52.94% of 17 liver samples.

Within the scope of the study, the rate of obtaining *P. multocida* from goat lung samples (1.85%) was determined to be lower than the data obtained by Özbey and Muz (2004), Ferdausi et al. (2008), Rashid et al. (2013), Jarikre et a.l (2018) and Baykan et al. (2023). The *M. haemolytica* isolation rate obtained in the study was found to be lower compared to the results of the studies conducted by Özbey and Muz (2004), Jarikre et al. (2018) and Baykan et al. (2023). Similarly, the *E. coli* isolation rate in this study was found to be lower compared to other studies [Özbey and Muz (2004), Ferdausi et al. (2008), Rashid et al. (2013), Jarikre et al. (2018), El-Mashad et al. (2019), Adam et al. (2023)].

On the other hand, a study conducted by Thakur et al. (2019) in India reported that they collected 50 samples (44 nasal swabs and 6 lung samples) from sheep and

goats and found *H. somni* in 1 (2%) goat nasal swab sample, *T. pyogenes* in 10 (20%), and *Mycoplasma* spp. in 11 (22%), but they could not identify *H. somni, T. pyogenes,* and *Mycoplasma* spp. from any of the samples collected from sheep. Similarly, a study conducted by Babu et al. (2019) in India reported the identification of *P. multocida* in 9.30%, *M. haemolytica* in 11.62%, and *E. coli* in 46.5% of 43 lung swab samples collected from sheep and goats.

As a result of the studies, it was observed that the isolation rates of *P. multocida, M. haemolytica, E. coli, Klebsiella* spp., *Klebsiella* spp., *T. pyogenes,* and *H. somni* from sheep and goat lung samples varied between 0-31.6%, 0-56.14%, 2.8-52.94%, 0.7-51.72%, 0-20%, and 0-8.7%, respectively. The identification rates of bacterial agents obtained as a result of this study are shown in Table 2. In this study, sheep and goat lung samples were tested negative for *H. somni.* in this study, *K. pneumonia* was not isolated in goats conventional and molecular methods.

This may be attributed to geographical differences, different regional climatic characteristics, as well as different sample sizes and diagnostic methods used. Furthermore, it was also considered that the detection of more parasitic infestations in the lung samples with macroscopic lesions (unpublished data) may have caused this condition. Moreover, it was concluded that the maintenance of the nomadic breeding style in Siirt region for most of the year may lower the bacterial pneumonia rates by allowing the animals to stay less in the cramped barn environment.

Antimicrobial resistance may develop in bacteria due to unnecessary and incorrect use of antibiotics used for the treatment of bacterial diseases. In such cases, it is possible to develop resistance not only in pathogenic bacteria but also in normal flora bacteria. Developed antimicrobial resistance causes significant yield and economic losses due to the prolonged treatment period of animals and the increase in treatment costs (Gulaydin et al. 2021).

There are national and international studies on the subject. Accordingly, Rashid et al. (2013) reported that E. coli isolates obtained from lung tissue samples with pneumonic lesions were sensitive to streptomycin and ciprofloxacin. Also, in their study, Babu et al. (2019) reported that 100% of *P. multocida* and *M. haemolytica* strains and 70% of *E. coli* strains were susceptible to enrofloxacin. A study conducted by Özavcı et al. (2022) reported that all *M. haemolytica* isolates and 90% of *P. multocida* isolates were susceptible to tulathromycin, florfenicol, and amoxicillin-clavulanic acid. In their study, Rashid et al. (2013) showed that the susceptibility rate of *E. coli* isolates to streptomycin in was higher than the results of this study, while a similar rate of susceptibility was found against ciprofloxacin. While the results of the study by Babu et al. (2019) indicated that susceptibility rates of *P. multocida* and *M. haemolytica* isolates to enrofloxacin were lower than those in this study, the results of the study conducted by Özavcı et al. (2022) showed that susceptibility rates of *P. multocida* and *M. haemolytica* isolates to tulathromycin, florfenicol, and enrofloxacin were lower than those in this study. It was determined that antimicrobial resistance rates of bacterial agents isolated from different regions may differ. It was considered that this may be attributed to the habits of antibiotic use among veterinarians or livestock breeders in the region.

CONCLUSION

Consequently, this study revealed the presence of *P. multocida*, *M. haemolytica*, *E. coli* and *T. pyogenes* which were the causative agents of pneumonia in sheep and goats, leading to significant yield losses, by using phenotypic and genotypic methods. Furthermore, based on antimicrobial susceptibility testing, the following antibiotics were found to be effective: cefpodoxime, ceftiofur, enrofloxacin, florfenicol, and spectinomycin for *P. multocida* and *M. haemolytica*; piperacillin/tazobactam, ertapenem, and cefoxitin for E. coli; and danofloxacin, amoxicillin/clavulanic acid, oxytetracycline, erythromycin, ceftiofur, florfenicol, and sulfamethoxazole+trimethoprim for *T. pyogenes*. Due to the frequent and indiscriminate use of antibiotics in pneumonia cases, multi-resistant strains are often detected. Antibiotic susceptibility varies between farms and bacterial agents. For this reason, it can be suggested that antibiotic sensitivity testings should definitely be carried out and antibiotics should be used according to the results.

In addition, in order to obtain reliable data, it was concluded that conventional bacteriological methods should be supported by molecular methods.

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RESEARCH ARTICLE

Assessment of DNA Damage and Oxidant/Antioxidant Balance After Acute Exposure to Glyphosate Isopropylamine in Rats

Ruhi TÜRKMEN[1], Yavuz Osman BİRDANE[1], Orkun ATİK[1]*

[1]*Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Türkiye*

ABSTRACT

In this study, the effects of oral administration of glyphosate isopropylamine (GI) salt at different doses (1/10 and 1/2 of LD50) on DNA damage, oxidant/antioxidant balance and some biochemical parameters were investigated to evaluate the acute toxicity of glyphosate isopropylamine salt. The results showed that 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels were significantly increased, malondialdehyde (MDA) levels were increased in all tissues except blood and heart and glutathione (GSH) levels were decreased in rats in the high dose group. In addition, significant increases in catalase (CAT) and superoxide dismutase (SOD) enzyme activities were detected. Biochemical analyses demonstrated that acute exposure to herbicides led to a significant elevation in serum alkaline phosphatase (ALP), albumin and urea levels, but no significant changes in AST, ALT, GGT, total protein (Tp), creatinine and CK-MB levels. These findings suggest that exposure to high doses of glyphosate may cause toxic effects by triggering oxidative stress and DNA damage.

Keywords: Acute exposure, Glyphosate isopropylamine, Rats

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Sıçanlarda Glifosat İzopropilamin Akut Maruziyeti Sonrası DNA hasarı ve Oksidan/Antioksidan Denge Değerlendirmesi

ÖZ

Bu çalışmada, glifosat izopropilamin (GI) tuzunun akut toksisitesini değerlendirmek amacıyla sıçanlara farklı dozlarda (LD50'nin 1/10 ve 1/2'si) oral olarak uygulanmasının, DNA hasarı, oksidan/antioksidan denge ve bazı biyokimyasal parametreler üzerindeki etkileri incelenmiştir. Sonuçlar, yüksek doz grubundaki sıçanlarda 8-hidroksi-2'-deoksiguanozin (8-OHdG) seviyelerinin belirgin şekilde arttığını, malondialdehit (MDA) düzeylerinin kan ve kalp dışındaki tüm dokularda yükseldiğini ve glutatyon (GSH) seviyelerinin ise azaldığını göstermiştir. Ayrıca, katalaz (CAT) ve süperoksit dismutaz (SOD) enzim aktivitelerinde anlamlı artışlar tespit edilmiştir. Biyokimyasal analizler, herbisite akut maruziyetin serum alkalen fosfataz (ALP), albümin ve üre seviyelerinde artışa yol açtığını ancak AST, ALT, GGT, total protein (Tp), kreatinin ve CK-MB seviyelerinde belirgin bir değişiklik oluşturmadığını ortaya koymuştur. Bu bulgular, yüksek dozda glifosata maruziyetin oksidatif stres ve DNA hasarını tetikleyerek toksik etkilere neden olabileceğini göstermektedir.

Anahtar Kelimeler: Akut maruziyet, Glifosat izopropilamin, Sıçan

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ORCID ID; *RT: 0000-0003-4726-3900, YOB: 0000-0003-0754-7909, OA: 0000-0003-2411-7492*
*Corresponding author e-mail: oatik@aku.edu.tr

GİRİŞ

Dünya nüfusunun hızla artması ve ekilebilir arazilerin daralması, gıda ihtiyacını daha da kritik hale getirmiştir. Bu durum, ekili bitkiler ve gıda ile yem depolarındaki kayıpları önlemek amacıyla pestisitler de dahil olmak üzere çeşitli kimyasalların kullanılmasına neden olmuştur. Ancak bu kimyasalların gelişigüzel kullanımı, hedef dışı organizmalara zarar vererek ve çevrede büyük değişikliklere neden olarak biyoçeşitliliği ciddi şekilde etkileyebilmektedir (Dar ve ark. 2019). Bu amaçla kullanılan pestisit miktarı Tarım ve Orman Bakanlığı'nın en son verilerine göre (2025a), 2006'da 45376 ton iken 2023'de 57766 ton; bu grubun içinde yer alan herbisitlerin oranı da 2006'da 6956 ton iken 2023 yılı için yaklaşık 16 bin ton olmuştur. Tarım ilacı kullanım miktarları gruplar bazında incelendiğinde fungusitlerin ardından 2. sırada herbisitler gelmektedir. Ayrıca, aktif madde düzeyinde Türkiye'de en yaygın kullanılan herbisitlerin başında glifosat izopropilamin (GI) tuzu yer almaktadır. Yapılan tespitlere göre, aktif durumda olan ruhsatlı ilaç sayısı 2702'dir (Tarım ve Orman Bakanlığı, 2025b).

Genellikle izopropilamin tuzu olarak formüle edilen glifosat, bir organofosfat (OF) bileşiği olup, geniş yapraklı yabani otları ve odunsu bitkileri hedef alan geniş spektrumlu sistemik bir herbisit veya yabani ot kurutucudur (Bou-Mitri ve ark. 2025). Tarım sektörü, 1970'lerden bu yana sağlıklı florayı öldürmeden zararlı yabani otları öldürmek için ağırlıklı olarak GI kullanmaktadır. Ayrıca, ev çimleri, bahçeler, parklar, golf sahaları ve yüzme havuzlarının rekreasyonu için artan talep, dünya çapında sıklık ve hacim olarak yüz kat daha fazla tüketimle sonuçlanmakta ve orantılı olarak daha fazla artış beklenmektedir. Yaygın kullanım ve kolay erişim kaçınılmaz olarak çevre kirliliğine ve büyük miktarda zehirlenmeye yol açmaktadır. Her yıl acil servise başvuran zehirlenmelerin en yaygın nedenidir. GI zehirlenmesi mağdurları, ölümcül bir sonuçtan veya yüz binlerce morbidite ve mortaliteden kaçınmak için hastanenin acil servisinde acil tedavi gerektirir (Basarslan ve Basarslan, 2023).

Glifosat tuzu herbisit içeren formülasyonu ile ilişkili ölümler ve kardiyovasküler yan etkilerin araştırıldığı bir çalışmada, GI grubunda amonyum grubuna göre daha fazla ölüm, daha yüksek QTc uzaması insidansı ve daha yüksek PR uzaması eğilimi görüldüğü rapor edilmiştir (Moon ve ark. 2018). Her ne kadar bitki ve memeli metabolizmasındaki önemli farklılıklar nedeniyle GI, insanlar için minimal toksik olarak kabul edilse de (Williams ve ark. 2000), giderek artan kanıtlar, toksisitesinin insanlarda önemsiz olmayabileceğini göstermektedir (Picetti ve ark. 2017; Kunapareddy ve Kalisetty, 2021). Bu durum, GI kaynaklı memeli toksisitesinde, bitkilerdeki etki mekanizmasından bağımsız olarak oksidatif stres ve DNA hasarı gibi farklı yolakların rol oynayabileceğini göstermektedir (Zanchi ve ark. 2024).

Serbest radikallerin biyolojik sistemlerde çeşitli faktörler nedeniyle birikmesinin yanı sıra, endojen antioksidanların bu radikalleri etkisizleştirme kapasitesindeki dengenin bozulması, oksidatif stres olarak tanımlanmaktadır (Halliwell ve Gutteridge, 2015). Oksidatif stresin ana ürünlerinden olan reaktif oksijen türleri (ROS), yüksek reaktiviteye sahip moleküller olup, mitokondri başta olmak üzere hücre organellerinde normal metabolik süreçlerin yanı sıra iskemi-reperfüzyon, yaşlanma, radyasyon, yüksek oksijen basıncı, inflamasyon ve pestisitler gibi ksenobiyotiklere maruz kalma gibi durumlar sonucunda üretilebilirler (Sies ve Jones, 2020). DNA hasarı genellikle öncelikle baz eksizyon onarım yolu ile onarılır ve oksitlenmiş ürünler idrarla atılır. 8-hidroksi-2-deoksiguanozin (8-OHdG) en yaygın olarak çalışılan oksitlenmiş metabolitlerden biridir ve DNA'nın oksidatif hasarı için bir biyobelirteç olarak kabul edilir (Graille ve ark. 2020). Son zamanlarda yapılan bazı çalışmalar, özellikle ROS ile ilgili olarak, pestisit patogenezinde hücre antioksidan kapasitesinin yetersizliğinin potansiyel etkilerine ve DNA hasarına dikkat çekmiştir (Alvarez-Moya ve ark. 2011; Turkmen ve ark. 2019a; Turkmen ve ark. 2019b; Basarslan ve Basarslan 2023; Smith-Roe ve ark. 2023). Örneğin, Turkmen ve ark. (2019a; 2019b), 375 mg/kg GI ile oral yoldan uygulanan Wistar sıçanlarının, serbest radikal aracılı lipid peroksidasyon (LPO) kaskadının stabil bir metaboliti ve oksidatif stresin bilinen bir belirteci olan malondialdehit (MDA) seviyelerinde artış gösterdiğini bildirmişlerdir. Benzer şekilde, tiyol grupları oksidatif hasara karşı hassastır ve bu tür bir hasar meydana geldiğinde, bu gruplar serbest radikalleri temizleyebilir. Glutatyon (GSH), bu tiyol gruplarından birini oluşturur ve serbest radikallerin temizlenmesi, elektrofilik bileşiklerin detoksifikasyonu ve hücresel redoks durumunun modüle edilmesinde vücudun doğal savunma sisteminin önemli bir bileşenidir. Bu nedenle GI tarafından indüklenen oksidatif stresin şiddeti bu tiyol grupların ölçülmesiyle belirlenebilir. Ayrıca, GI ile ilişkili oksidatif stres, organizmayı toksisitenin etkilerine karşı savunmada görevli süperoksit dismutaz (SOD) ve katalaz (CAT) gibi antioksidan enzimlerin aktivitesini yavaşlatabilir. Bu hücre içi antioksidan enzim aktivitelerinin ölçülmesi de GI ile ilişkili oksidatif hasarın şiddetini ortaya koyabilir. Chen ve ark. 2022).

GI'nın vital organlarda oksidatif stresi ve DNA hasarını indüklediği birçok çalışmada doğrulanmıştır (Mazuryk ve ark. 2024a; Mazuryk ve ark. 2024b). Ancak bu çalışmalarda kullanılan ticari ürünler ve dozlar, bizim çalışmamızdakilerden farklılık göstermektedir. Bu çalışmada kullanılan %48 oranında glifosat ve sürfektan içeren Knockdown® SL, GI tuzunun bir ticari formudur ve dünyada olduğu gibi Türkiye'de de yaygın olarak kullanılmaktadır. Daha önceki bir çalışmamızda bu

kimyasalın öldürücü dozunun (LD_{50}) 7878,5 mg/kg olduğu belirlenmişti (Turkmen ve Dogan 2020). GI'nın yüksek ($LD_{50}/2$) ve düşük ($LD_{50}/10$) dozlarının akut maruziyetine bağlı toksik potansiyeli hakkında çalışma bulunmamaktadır. Özellikle doku oksidatif stresinin boyutuyla ilgili veriler eksiktir. Bu bilgiler ışığında çalışmanın amacı, GI tuzunun yüksek ($LD_{50}/2$) ve düşük ($LD_{50}/10$) iki oral dozunun akut maruziyet sonrası sıçan kan, beyin, kalp, karaciğer, böbrek ve testis dokularında oksidan/antioksidan denge, DNA hasarı ve bazı biyokimyasal parametreler üzerindeki etkisini araştırmaktır.

MATERYAL ve METOT

Kimyasal

GI (Knockdown 48 SL), HEKTAŞ (Kocaeli, Türkiye) şirketinden satın alındı. Hayvanlara verilen GI dozu Turkmen ve Dogan (2020) tarafından yapılan çalışma dikkate alınarak belirlendi.

Hayvan Materyali

Hayvan materyali olarak, onsekiz adet yetişkin (180-200 g ağırlığında ve 2,5-3 aylık) erkek Wistar albino sıçan, Afyon Kocatepe Üniversitesi Deney Hayvanları Araştırma ve Uygulama Merkezi'nden temin edildi. Bir haftalık adaptasyonun ardından, üç gruba randomize edilen hayvanlar (n = 6), kontrollü bir ortamda (22 °C, 12 saat aydınlık-karanlık döngüsü) serbest yiyecek ve su ile muhafaza edildi. Çalışmaya, Afyon Kocatepe Üniversite Hayvan Deneyleri Yerel Etik Kurulu tarafından izin verildi (AKUHADYEK, Ref No: 94/19, Tarih: 09/2019).

Deneysel Uygulamalar

1. Grup: Kontrol; oral gavaj ile distile su verildi.
2. Grup: Doz 1 (D1); oral gavaj ile GI'nın $LD_{50}/2$ dozu (tek uygulama) verildi.
3. Grup: Doz 2 (D2); oral gavaj ile GI'nın $LD_{50}/10$ dozu (tek uygulama) verildi.

Örneklerin Toplanması ve Analizi

İlaç uygulamasından 24 saat sonra ketamin/ksilazin anestezisi altında sıçanların göğüs boşluğu açılarak kardiyak punksiyon ile heparinli ve normal tüplere kan alındı. Anestezi altındaki sıçanlar eksanguinasyon ile sakrifiye edildi. Heparinli tüplere alınan kanların 1 ml'si MDA ve GSH analizleri için ayrıldı ve aynı gün çalışıldı. Geri kalan kanlardan eritrositlerin hazırlanması Winterbourn ve ark. (1975) yöntemine göre gerçekleştirildi ve SOD ile CAT analizleri eritrosit lizatında çalışıldı. Normal tüpe alınan kandan serum ayrıldı. Daha sonra, karaciğer, böbrek, kalp, beyin ve testis dokularının bir kısmı, biyokimyasal analizler için 0.15 M Tris-HCl tamponunda (pH 7.4) homojenize edildi. Hoöojenizasyon işleminden sonra dokular 4°C'de 2500 g'de 10 dk santrifüj edildi ve elde edilen süpernatantlar analiz yapılıncaya kadar 20°C'de saklandı.

Tam kan ve doku homojenatlarının MDA (Draper ve Hardley 1990; Ohkawa vd. 1979) ve GSH (Beutler vd. 1963) düzeyleri spektrofotometrik yöntemlerle belirlendi. Ayrıca, eritrosit lizatı ve doku homojenatının SOD (Sun et al. 1988) ve CAT (Luck 1955; Aebi 1974) aktiviteleri spektrofotometrik olarak ölçüldü. Hemoglobin ve dokuların protein içerikleri sırasıyla Drabkin ve Austin (1935) ve Lowry ve arkadaşlarının (1951) yöntemlerine göre analiz edildi.

Serum 8-OHdG Düzeylerinin Belirlenmesi

DNA oksidasyon belirteci olarak 8-hidroksi-2'-deoksiguanozin (8-OhdG) seviyelerinin ölçümü, ELABSCIENCE Şirketinin ELISA (Katalog numarası: E-EL-0028) kiti kullanılarak belirlendi.

Serum Biyokimyasal Parametrelerinin Analizi

Serumdan aspartat aminotransferaz (AST), alkalen fosfataz (ALP), alanin aminotransferaz (ALT), gama glutamin transferaz (GGT), albümin, total protein (Tp), üre, kreatinin ve kreatin kinaz (CK-MB) ölçümü otoanalizör cihazı yardımıyla yapıldı. Bu belirteçlerin serum düzeyleri Abbott Architect c8000 cihazında Abbott kitleri kullanılarak belirlendi.

İstatistiksel Analiz

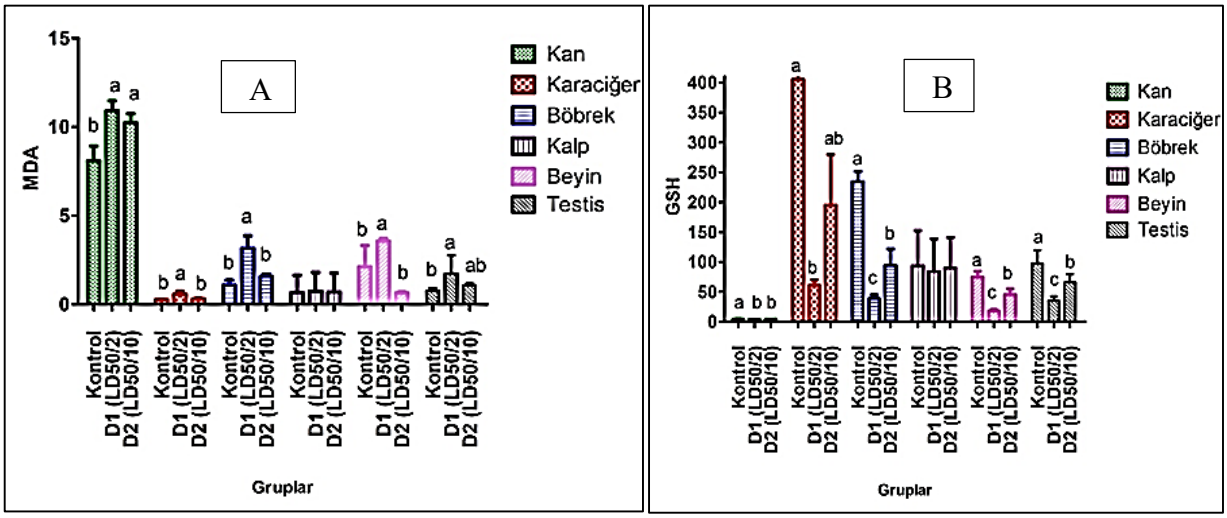
Sonuçlar, ortalama ± ortalamanın standart hatası (SEM) olarak ifade edildi. Verilerin normalliği ve homojenliği sırasıyla Kolmogorov-Smirnov ve Levene testleri kullanılarak kontrol edildi. Normal dağılım gösteren veri setleri için tek yönlü varyans analizi (ANOVA) uygulandı ve gruplar arası farklılıklar Duncan'ın post hoc testi kullanılarak belirlendi. Analizlerde anlamlılık düzeyi $p<0.05$ olarak belirlenmiştir. Tüm veriler Windows için Prism 5 (sürüm 5.03) istatistiksel yazılım paketi (GraphPad Software Inc., La Jolla, CA, ABD) kullanılarak analiz edilmiştir.

BULGULAR

Akut GIifosat izopropilamin (GI) Maruziyetinin MDA ve GSH Düzeylerine Etkisi

Sonuçlar, Şekil 1A (MDA) ve Şekil 1B (GSH)'de gösterildi. MDA düzeyleri bakımından gruplar incelendiğinde, kontrol grubuna göre kalp dışındaki tüm organlarda ve kanda özellikle yüksek doz herbisit uygulanan hayvanlarda MDA düzeyleri daha yüksekti ($p<0,05$).

GSH seviyeleri bakımından gruplar incelendiğinde ise kontrol grubuna kıyasla kalp dışındaki tüm organlarda özellikle yüksek doz herbisit uygulanan hayvanlarda GSH seviyeleri oldukça düşüktü ($p<0.05$).

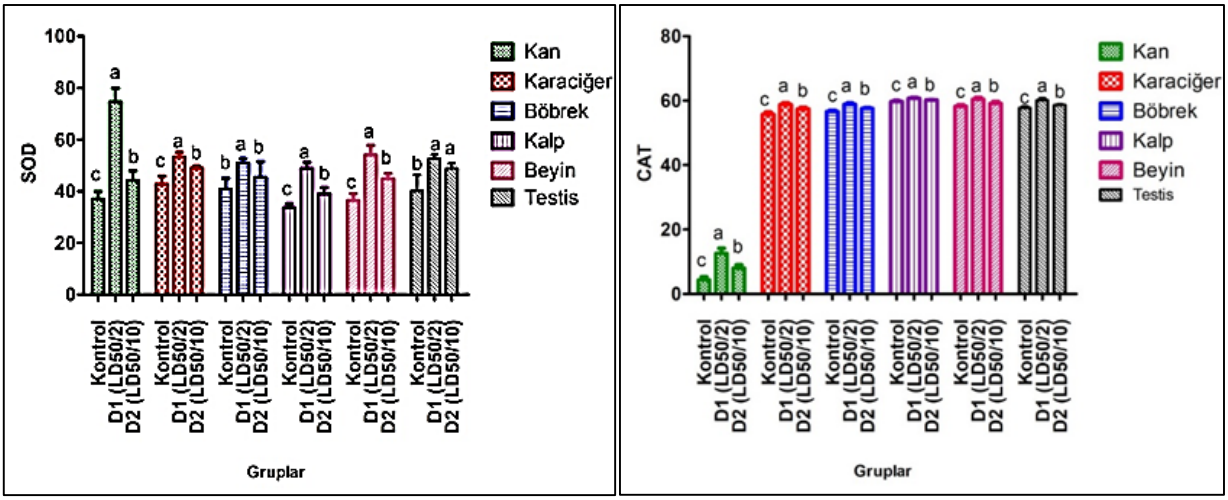


Şekil 1: Glifosat izopropilaminin farklı dozlarına (D1, LD50/2 ve D2, LD50/10) oral yoldan maruz bırakılan sıçanların farklı organlarındaki MDA (A) ve GSH (B) seviyelerinin bar grafikleri. Grafikte farklı harflerle gösterilen değerler istatistiksel olarak anlamlıdır ($p<0,05$).

Figure 1: Bar graphics of the MDA (A) and GSH (B) levels in different organs of rats exposed orally to different doses (D1, LD50/2 ve D2, LD50/10) of glyphosate isopropylamine. The values shown with different letters in the graph are statistically significant ($p<0.05$).

Akut GIifosat izopropilamin (GI) Maruziyetinin SOD ve CAT Aktivitelerine Etkisi

Sonuçlar, Şekil 2A (SOD) ve Şekil 2B (CAT)'de gösterildi. Kontrol ile karşılaştırıldığında hem eritrositlerde hem de tüm organlarda, özellikle de yüksek herbisit dozlarına maruz kalan hayvanlarda, SOD ve CAT gibi antioksidan enzimlerin aktivitelerinde önemli artışlar olduğu görüldü ($p<0,05$).



Şekil 2. Glifosat izopropilaminin farklı dozlarına (D1, LD50/2 ve D2, LD50/10) oral yoldan maruz bırakılan sıçanların farklı organlarındaki SOD (A) ve CAT (B) aktivitelerinin bar grafikleri. Grafikte farklı harflerle gösterilen değerler istatistiksel olarak anlamlıdır ($p<0,05$).

Figure 2. Bar graphics of the SOD (A) and CAT (B)activities in different organs of rats exposed orally to different doses (D1, LD50/2 ve D2, LD50/10) of glyphosate isopropylamine. The values shown with different letters in the graph are statistically significant ($p<0.05$).

Akut GIifosat İzopropilamin (GI) Maruziyetinin Bazı Biyokimyasal Parametrelere Etkisi

Karaciğer fonksiyon parametreleri AST, ALT, ALP, GGT, Tp ve albümin; böbrek fonksiyon parametreleri üre, kreatinin aktiviteleri ve kalp biyomarkırı CK-MB düzeyleri üzerine akut GI maruziyet sonuçları Tablo 1.'de sunuldu. Kontrol grubuna göre karşılaştırıldığında, herbisite akut maruz kalmanın serum ALP, albumin ve üre seviyelerinde önemli bir artışa neden olduğu görüldü ($p<0,05$). Bununla birlikte gruplar arasında serum total protein, AST, ALT, GGT, kreatinin ve CK-MB seviyelerinde istatiksel anlamda önemli bir değişiklik olmadı ($p>0,05$).

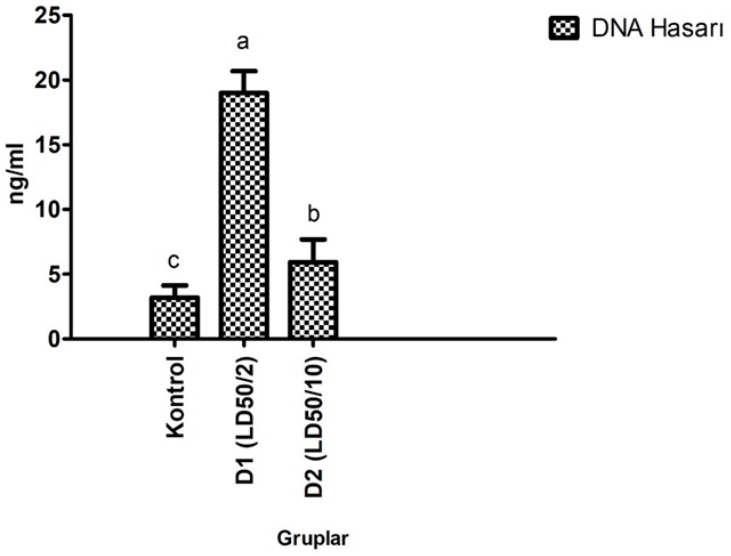
Tablo 1. Akut gIifosat izopropilamin (GI) maruziyetinin bazı biyokimyasal parametrelere etkisi
Table 1. Effects of acute gIyphosate isopropylamine (GI) exposure on some biochemical parameters

Gruplar ve Parametreler	Kontrol	D1 (LD50/2)	D2 (LD50/10)	*P* değeri
Tp (g/dL)	5,18±0,27	5,62±0,54	5,34±0,23	0,164
AST (U/L)	158,00±15,22	159,67±32,76	174,33±41,16	0,625
ALT (U/L)	63,83±14,62	84,50±11,48	68,67±15,64	0,054
ALP (U/L)	376,67±126,74[c]	579,67±84,97[a]	411±84,08[b]	**0,007**
GGT (U/L)	5,33±4,46	2,83±4,12	2,33±2,58	0,368
Albümin (g/dL)	3,08±0,13[b]	3,33±0,11[a]	3,16±0,20[a]	**0,035**
Üre (mg/dL)	42,60±5,35[b]	51,43±6,31[a]	53,53±5,66[a]	**0,012**
Kreatinin (mg/dL)	0,44 ± 0,06	0,46±0,12	0,40±0,08	0,463
CK-MB (IU/L)	654,83±223,31	756,33±175,21	614,83±242,93	0,519

Mean ± SEM; n=6
a,b,c: Aynı satırda farklı harflerle gösterilen değerler istatistiksel olarak anlamlıdır (p<0,05).

Akut GIifosat İzopropilamin (GI) Maruziyetinin DNA Hasarına Etkisi

Sonuçlar, Şekil 3.'de gösterildi. 8-OHdG düzeyleri bakımından gruplar incelendiğinde, kontrol grubuna göre herbisitin her iki dozuna da maruz kalan hayvanlarda 8-OHdG düzeyleri anlamlı derecede yüksekti (p<0,05).



Şekil 3: Glifosat izopropilaminin farklı dozlarına (D1, LD50/2 ve D2, LD50/10) oral yoldan maruz bırakılan sıçanların serum 8-OHdG düzeylerinin bar grafikleri. Grafikte farklı harflerle gösterilen değerler istatistiksel olarak anlamlıdır (p<0,05).
Figure 3: Bar graphics of the 8-OHdG levels in serum of rats exposed orally to different doses (D1, LD50/2 ve D2, LD50/10) of glyphosate isopropylamine. The values shown with different letters in the graph are statistically significant (p<0.05).

TARTIŞMA

Bu çalışma, akut GI maruziyetine bağlı oksidatif stres ve DNA hasarının boyutunu biyokimyasal, oksidan-antioksidan ve 8-OHdG analizleriyle ortaya koymuştur.

Aerobik yaşam ve enerji metabolizması süreçleri nedeniyle, canlı organizmalar belirli bir düzeyde oksidatif hasara maruz kalmaktadır. Oksidatif stresin derecesi, serbest radikaller ve reaktif oksijen ile azot türlerinin üretim hızına ve organizmanın sahip olduğu antioksidan savunma mekanizmalarının etkinliğine bağlıdır (Bayezit ve Kart 2021). Bazı biyokimyasal parametrelerde gözlenen değişiklikler, sıçanların GI'ya maruz kalmasının kan ve farklı dokularda (karaciğer, böbrek, beyin, testis) LPO'yu önemli ölçüde artırdığını ve böylece LPO'nun akut OF kaynaklı toksisitede yer alan moleküler mekanizmalardan biri olabileceği hipotezini desteklediğini göstermektedir (Sule ve ark. 2022). Antioksidan sisteminin bozulması, hücre zarında hasara, zarın akışkanlık ve geçirgenliğinde değişikliklere yol açabilir; bu durum ise oksidatif stresin ortaya çıkmasına ve örneğin DNA hasarına sebep olabilir (Hernández-Moreno ve ark. 2018).

LPO'nun bir ürünü ve belirteci olan MDA, oldukça reaktif bir metabolittir (Khan ve Rampal 2014). Bu nedenle, artan LPO seviyeleri, süperoksit, hidrojen peroksit ve hidroksil radikallerinin birikmesine yol açar ve bu da LPO'nun daha da kötüleşmesine neden olur (Noeman ve ark. 2011). Aşırı serbest radikal oluşumunu kompanze edebilmek için antioksidan enzimlerin aktivitesinde meydana gelen artış, bizim bulgularımızla uyumlu bir şekilde, hücresel savunma mekanizmalarının güçlendiğini göstermektedir. Önceki araştırmalarda, GI uygulamasının insan, sıçan ve farelerde MDA seviyelerini artırdığı tespit edilmiştir. (Pieniążek ve ark. 2004; Astiz ve ark., 2009; Cavuşoğlu ve ark., 2011). Bu çalışmada da, kalp dokusu hariç diğer tüm dokularda MDA seviyelerinin arttığı, en yüksek MDA seviyelerinin ise kanda gözlendiği tespit edilmiştir. Bu durum, akut maruziyet sonucunda kana salınan yüksek miktardaki serbest radikallerden kaynaklanıyor olabilir.

Oksidasyon kaynaklı hasarı önlemek için organizmaların etkili antioksidan sistemlere sahip olması gerekir. Bu sistemlerin bazı bileşenleri, indirgenmiş glutatyon (GSH) ve glutatyon peroksidaz veya katalaz gibi serbest radikal temizleyici enzimlerin yanı sıra GSH geri dönüşümünde önemli bir role sahip olan glutatyon redüktaz (GR) gibi enzimler de dahil olmak üzere bazı antioksidan enzimleri içerir. GSH, hücre içindeki antioksidan savunma sisteminin bir bileşeni olup, diazinon veya atrazin gibi çeşitli ksenobiyotiklere maruz kalmanın ileriye dönük biyolojik göstergesi olarak kullanılabilir (De La Casa-Resino ve ark. 2013; Hernández-Moreno ve ark. 2018). Aynı zamanda hücre içi redoks tampon sisteminin ana bileşenidir. Pestisitler gibi ksenobiyotikler ile reaksiyona girerek kompleks formlar oluştururlar. GSH, bu şekilde hücreleri ksenobiyotiklerin toksik etkilerinden korur (Chhabra ve ark. 1993). Oksidatif stres durumlarında artmış LPO bağlı olarak meydana gelen peroksitlerin detoksifikasyonunda görev alan enzimler tarafından GSH tüketilir (Cathcart 1985). Bu çalışmada kalp dokusu hariç diğer tüm dokularda GSH seviyelerinin azaldığı gözlendi. Bu durum GSH'nun GI uygulanması sonucu ortaya çıkan serbest radikaller ve peroksitlerle reaksiyona girerek düzeyinin azalabileceği şeklinde yorumlanmaktadır.

Normal fizyolojik şartlarda hem SOD hem de CAT gibi hücre içi antioksidan enzimler serbest radikalleri ortadan kaldırarak hücrelerin antioksidan savunma sisteminde bütünleyici bir rol oynarlar (Bukowska, 2004). SOD, süperoksit radikalleri H_2O_2'e dönüşümünü katalize ederken, CAT H_2O_2'i suya dönüştürür. Bu antioksidan enzimler bu yüzden serbest radikallerin toksik etkilerini azaltabilir (Mansour ve Mossa, 2009). Farklı pestisitlerle yapılan çalışmalarda pestisitlerin SOD ve CAT gibi antioksidan enzimlerin aktivitelerine etkisi konusunda çelişkili ifadeler bulunmaktadır. Kanbur ve ark. (2009) yaptıkları çalışmada organik fosforlu bir pestisid olan propetamfosun rat eritrosit SOD ve CAT aktivitelerini azalttığını bildirmişlerdir. Ince ve ark. (2017) ise malatiyon tarafından oluşturulan oksidatif hasar neticesinde rat eritrosit ve dokularında SOD ve CAT aktivitelerinin arttığını rapor etmişlerdir. Bizim çalışmamızda da akut GI'ya maruz bırakılan ratlarda, eritrosit, karaciğer, kalp, böbrek, beyin ve testis dokularında SOD ve CAT aktivitelerinin azaldığı görüldü. GI grubunda SOD ve CAT aktivitelerinin düşük bulunması oksidatif stresin artışına bağlı olarak bu enzimlerin tükenmesi ile ilişkili olabilir.

Karaciğer, bir hayvanın vücudunda hayati ve dirençli bir organdır ve temel işlevleri metabolizmayı, sindirimi, detoksifikasyonu, vitaminleri, mineralleri ve bağışıklığı depolamaktır. Ancak bu organ gıda takviyeleri, ilaçlar, kimyasallar ve şifalı bitkilerden kaynaklanan toksinlerden etkilenebilir (Thompson ve ark. 2017). Ayrıca glifosat içeren herbisitlerin bir çok çalışmada nefrotoksisiteye neden oldukları öne sürülmektedir (Wunnapuk ve ark. 2014; Gao ve ark. 2019; Trasande ve ark. 2020). Ek olarak GI'nın yutulması veya kendi kendini zehirleme durumunda, sistemik glifosat konsantrasyonunun yüksek olması beklenir. Kasıtlı veya kazara oral alımdan sonra akut kendi kendine zehirlenme ölümcül olabilir ve en ciddi şekilde zehirlenen hastalar akut solunum sıkıntısı, nörolojik ve şiddetli gastrointestinal semptomlar gibi diğer organ komplikasyonlarının eşlik ettiği akut böbrek yetmezliğinden ölür ve kusma ve ishal nedeniyle aşırı sıvı kaybı hipovolemik şokla sonuçlanır (Roberts ve ark. 2010). Karaciğer, böbrek ve kalp fonksiyon testlerini değerlendirmek için, karaciğer için AST, ALT, ALP, GGT, Tp, ve albümin; böbrek için üre, kreatinin ve kalp için CK-MB gibi bazı önemli

belirteçler gruplar arasında değerlendirilmiştir. Bu testlerde, GI kaynaklı hepato-nefrotoksisitenin karaciğer ve böbrek enzimlerinin dolaşıma sızmasına ve serum enzim seviyelerinin yükselmesine neden olduğunu göstermiştir. Yukarıda belirtilen biyobelirteçlerin serumdaki artışına ilişkin bulgularımız, glifosat bazlı herbisitin akut ve kronik uygulanarak önemli serum enzimlerinin artışını gösteren diğer çalışmalarla (Jasper ve ark. 2012; Fadel ve ark. 2022) uyumludur.

Bildiğimiz kadarıyla, mesleki glifosat maruziyetini, ROS'a yanıt olarak oluşan ve pro-mutajenik bir DNA lezyonu olan 8-OHdG ile ilişkilendiren yalnızca bir çalışma bulunmaktadır (Koureas ve ark. 2014). Yunanistan'daki bir tarım topluluğunda 80 pestisit püskürtücüsü arasında, son ilaçlama sezonunda en az bir kez glifosat uygulayanların tam kanda yüksek 8-OHdG düzeylerine (>%75) sahip olma olasılığı, uygulamayanlara göre 1,5 kat daha fazladır; ancak bu ilişki tek değişkenli analize dayanmaktadır ve istatistiksel olarak anlamlı değildir (Koureas ve ark. 2014). EPA veya IARC tarafından değerlendirilen in vivo çalışmaların çoğu, GBH maruziyetini takiben tavşanlarda ve farelerde gösterilen mikronükleus indüksiyonu, kromozomal sapma ve DNA hasarının diğer yönlerini analiz etmiştir (Helal ve Moussa 2005; Amer ve ark. 2006; Benbrook 2019). Bu çalışmada, GI'nın düşük ve yüksek dozlarına akut maruziyet sonucu, oksidatif DNA hasarının biyobelirteci olan 8-OHdG seviyelerinin arttığı gözlemlenmiştir.

Sonuç olarak, GI'ya özellikle yüksek dozlarda maruz kalan sıçanlarda toksik etkiler olabileceği gösterilmiştir. Bununla birlikte glifosat formülasyonlarının şeffaf olmaması, kullanımının sağlık üzerindeki etkilerini anlamada zorluklara neden olmakta ve küresel verilerdeki boşluklarla birlikte düşünüldüğünde, bu kimyasalın hayvanlar ve insan popülasyonları üzerindeki çalışmalarını tutarsız hale getirmektedir. Bu nedenle, glifosat ve/veya glifosat bazlı herbisitlerin çevremiz ve sağlığımız üzerindeki gerçek etkilerini anlamaya devam etmek için daha fazla araştırmaya ihtiyaç vardır.

Çıkar çatışması: Yazarların bildirecekleri herhangi bir çıkar çatışması bulunmamaktadır.

Yazarların Katkıları: RT, YOB ve OA proje fikrine, çalışmanın tasarımına ve yürütülmesine katkıda bulunmuştur. RT ve OA veri toplama sürecine katkıda bulunmuştur. RT ve OA verileri analiz etmiştir. RT makale taslağını hazırlamış ve yazmıştır. RT, YOB ve OA makaleyi eleştirel bir gözle gözden geçirmiştir. Makalenin son hali tüm yazarlar tarafından okunmuş ve onaylanmıştır.

Etik onay: Bu çalışma Afyon Kocatepe Üniversitesi Araştırma Hayvanları Uygulama Merkezi'nde gerçekleştirilmiştir. Bu araştırma Afyon Kocatepe Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu tarafından onaylanmıştır (AKUHADYEK, Ref No: 94/19, Tarih: 09/2019).

Teşekkür: Bu çalışmada emeği geçen Afyon Kocatepe Üniversitesi Deney Hayvanları Araştırma ve Uygulama Merkezi çalışanlarına teşekkür ederiz.

Açıklama: Bu çalışmanın bir kısmı I. Uluslararası VI. Veteriner Farmakoloji ve Toksikoloji Kongresi'nde (2019) sözlü olarak sunulmuştur.

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RESEARCH ARTICLE

Comparison of Ultrasonographic and Laboratory Findings in Feline Lower Urinary Tract Disease

Ender ERKOÇ[1], Turan CİVELEK[2]*

[1]*Pasteur Veterinary Policlinic, Kocaeli, Türkiye*

[2]*Department of Internal Medicine, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Türkiye*

ABSTRACT

The aim of this study was to compare ultrasonographic and laboratory findings in cases of lower urinary tract disease diagnosed in cats randomly brought to the clinic with urinary tract complaints. The study material consisted of 50 cats of different breeds, age and sex diagnosed with feline lower urinary tract disease. The diagnosis was based on anamnesis, clinical findings, urine and biochemical analyses and ultrasonographic findings. Serum biochemistry and urine analysis results and ultrasonographic examination data were evaluated comparatively. The results of the study emphasise that cases of lower urinary tract disease in cats are most frequently encountered in male individuals and that there are significant differences between breeds. In the study, the most common problems in 50 cats diagnosed with lower urinary tract disease were struvite crystalluria; 30% and idiopathic cystitis; 28%, respectively. The data obtained revealed a significant positive moderate correlation between resistive index and serum phosphorus values and a significant positive moderate correlation between resistive index and blood-urea nitrogen and creatinine. There was also a significant positive moderate correlation between resistive index and urine protein values. The mean serum blood-urea nitrogen, creatinine and phosphorus concentrations measured in cats with lower urinary tract disease were relatively high, although there were individual differences between the cases. However, it is noteworthy that the mean blood urea-nitrogen and creatinine values were within normal limits, although there were individual differences, especially in cases of partial obstruction. One-month follow-up showed that the blood urea-nitrogen, creatinine and phosphorus values measured at the first presentation to the clinic were higher in cats that died than in surviving animals. The data presented in this study also revealed the presence of a positive significant correlation between urinary protein and serum blood urea-nitrogen and phosphorus values. The ultrasonographic renal measurement data evaluated in this master thesis study were recorded within the average normal values, although there were individual differences between the subjects. The mean resistive index value measured was below the upper limit of normal of 0.7. The results emphasise the importance of comparative evaluation of all measurement data in the diagnosis, patient follow-up, prognosis and determination of the correct treatment method in cases of feline lower urinary tract disease.

Keywords: Feline, FLUTD, Kidney, Resistive index, Urinalysis, USG

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Kedi Alt Üriner Sistem Hastalığında Ultrasonografik ve Laboratuvar Bulguların Karşılaştırılması

ÖZ

Bu çalışmada idrar yolu şikâyeti ile randomize olarak kliniğe getirilen kedilerde teşhis edilen alt üriner sistem hastalığı olgularında ultrasonografik ve laboratuvar bulgularının karşılaştırılması amaçlandı. Çalışma materyalini alt üriner sistem hastalığı tanısı alan, farklı ırk, yaş ve cinsiyette 50 kedi oluşturdu. Tanı; anemnez, klinik bulgular, idrar ve biyokimyasal analiz sonuçları ve ultrasonografi bulgularıyla kondu. Serum biyokimya ve idrar analiz sonuçları ve ultrasonografik muayene verileri karşılaştırmalı olarak değerlendirildi. Çalışma sonuçları kedilerde alt üriner sistem hastalığı olgularına en sık erkek bireylerde rastlandığına ve yine ırklar arası belirgin farkların olduğuna vurgu yapmaktadır. Çalışmada alt üriner sistem hastalığı tanısı alan 50 kedide en sık rastlanan promlemler, sırasıyla, strüvit kristalüri; %30 ve idiyopatik sistit; %28 olarak belirlendi. Elde edilen veriler; rezistif indeks ile serum fosfor değeri arasında pozitif orta düzeyde ve rezistif inseks ile kan-üre nitrojen ve kreatinin arasında ise pozitif orta düzeye yakın anlamlı bir ilişki ortaya koydu. Rezistif indeks ve idrar protein değerleri arasında da pozitif orta düzeye yakın anlamlı bir ilişki belirlendi. Alt üriner sistem hastalığı olan kedilerde ölçülen ortalama serum kan-üre nitrojen, kreatinin ve fosfor konsantrasyonlarının, olgular arasında bireysel farklar olmakla birlikte, nispi olarak yüksek olduğu gözlendi. Bununla birlikte; özellikle kısmi obstrüksiyonun gözlendiği vakalarda ortalama kan üre-nitrojen ve kreatinin değerlerinin, bireysel farklar olmakla birlikte, normal sınırlarda belirlenmesi dikkat çekicidir. Bir ay süreli gerçekleştirilen takip sonrası öldüğü belirlenen kedilerde, kliniğe ilk başvuruda ölçülen kan üre-nitrojen, kreatinin ve fosfor değerlerinin, sağkalan hayvanlara göre daha yüksek olduğunu belirlendi. Sunulan araştırma verileri, aynı zamanda, idrar protein ile serum kan üre-nitrojen ve fosfor değerleri arasında pozitif anlamlı bir ilişkinin varlığını da ortaya koydu. Bu yüksek lisans tez çalışmasında değerlendirmeye alınan ultrasonografik renal ölçüm verileri, olgular arasında bireysel farklar olsa da, ortalama normal değerler arasında kaydedildi. Ölçülen ortalama rezistif indeks değeri normal üst sınır olarak belirtilen 0.7'nin altındaydı. Sonuçlar; kedi alt üriner sistem hastalığı olgularında tanıda, hasta takibinde, prognozun tayininde ve doğru tedavi yönteminin tespitinde tüm ölçüm verilerinin karşılaştırmalı değerlendirmesinin önemine vurgu yapmaktadır.

Anahtar sözcükler: Böbrek, FLUTD, Kedigiller, Rezistif indeks, Urinalysis, USG

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ORCID ID; *EE: 0000-0003-1703-6950, TC: 0000-0002-4819-3484*

*Corresponding author e-mail: tcivelek@aku.edu.tr

INTRODUCTION

Feline Lower Urinary Tract Disease (FLUTD) is a common syndrome in veterinary medicine (Widyawati, et.al., 2022). It is found in cats of all ages and breeds. It is frequently seen in middle-aged, overweight, neutered male cats. Indoor cats that are not mobile, restricted to outdoor environment and fed with dry food are in the risk group (Guun-Moore, 2003).

Current studies classify FLUTD into two categories: FLUTD of unknown cause (idiopathic) and FLUTD of known cause. Urethral obstruction is commonly seen in 18-58% of cases during FLUTD. Therefore, FLUTD is also divided into obstructive FLUTD and non-obstructive FLUTD (Ay et al., 2021; Sabino, 2017; Savik et al., 2011 and Woolf, 2012). During this disease, idiopathic cystitis (FIC), urolithiasis, urethral plaque, tumour, anatomical defects, behavioural disorders, urinary tract infections (UTI) are frequently encountered in cats. The clinical symptoms of FLUTD include one or more of the following: pollakuria, dysuria, haematuria, stranguria, periuria (Forrester and Roudebush, 2007). In cats brought to the clinic with urinary system problems, correct diagnosis is extremely important for the prognosis of the disease. Imaging is important in diagnosis. For this purpose, x-ray and ultrasonography are used. Complete blood count, serum biochemistry, urine analysis, microscopic examination are other basic and auxiliary diagnostic methods (Hostutler et al., 2005).

MATERIALS and METHODS

Animal Material

The animal material of this study consisted of 50 cats with a mean age of 6.322±0.658 (Mean±SE), 21 females and 29 males, randomly admitted to the clinic with urinary tract complaints and diagnosed as FLUTD. No breed, sex and age differences were sought in the selection of the material. The study was performed at Pasteur Veterinary Polyclinic (Kocaeli, Turkey). The main complaints recorded in the anamnesis of the patients were pain during urination, blood in the urine, constant urge to urinate, spending too much time in the litter box, urinary incontinence and urinating in different areas. All cats included in the study were clinically diagnosed with FLUTD. Informed consent form was obtained from each owner.

For the research, Afyon Kocatepe University Animal Experiments Local Ethics Committee (AKÜ-HADYEK) was applied and necessary permissions (04.10.2022 date and 99 number) were obtained. This article was published in Veterinarian Ender Erkoç's Master's Thesis with the same title (AKÜ, Institute of Health Sciences, Thesis no: 869155, 2024/016).

Devices and Equipment

Haematological evaluations were performed with Mindray BC 5000 Vet, biochemical tests were performed with Fujifilm Dri-Chem NX500V, and complete urinalysis was performed with Vetscan UR devices. Philips Affiniti 50 colour doppler device was used for ultrasonographic imaging. Urine samples were collected with Buster sterile cat urinary catheter (1.0x130 mm) or cytocentesis method. Microscopic examination of urine was performed with Abaxis HD microscope, VD6M HD Camera w/hd.

Method

Urine Analysis

The collected urine samples were examined macroscopically for colour and then pH, leukocyte, protein, blood and nitrite parameters were evaluated by strip examination. The samples were centrifuged at 5000 rpm for 5 min and microscopic examination was performed according to the technique. In microscopic examination, the samples were evaluated for the presence of erythrocytes, leucocytes, epithelium and crystals.

Collection of Blood Samples

Blood samples were collected from the vena cephalica antebrahium into tubes containing EDTA for haematological examination and tubes containing gel-clot activator for biochemical analyses. Haemogram and biochemical analyses were performed simultaneously, without waiting.

Haematological Analysis

Leukocyte (WBC), erythrocyte (RBC), haemotocrit (HCT), platelet (PLT) values were measured with an automatic blood counting device in blood samples taken in EDTA tubes.

Biochemistry Analysis

Blood sera obtained from the samples taken in tubes containing gel-clot activator were analysed for blood urea nitrogen (BUN), creatinine (CREA), potassium (K), phosphorus (P) and sodium (Na) using a biochemistry autoanalyzer.

Imaging Methods

5-8 Mhz microconvex probe was used in the study. Images were recorded in longitinal and ventradorsal position. Patients were not sedated to prevent any effect on ultrasonography results. Renal arterial resistive index (RI) and renal aspect-height measurement, renal volume and cortex/medulla ratio were determined using colour and PW Doppler methods in accordance with the technique.

Survival

Patients were followed up for one month after the treatment procedure to determine the prognosis. The status at the end of one month was recorded as recovered (3), ongoing/follow-up (2) and lost (1).

Statistical Analyses

SPSS 21.0 windows package programme was used to analyse the data obtained in the study. Firstly, frequency, percentage, arrhythmic mean and standard deviation values were given for descriptive statistics. The relationships between the parameters were presented by Pearson correlation method. ANOVA (one-factor analysis of variance) was used to compare these parameters according to survival status. Statistical significance level was determined as $p<0.05$.

RESULTS

Evaluation of Clinical Findings

The breed and sex distribution of 50 cats diagnosed with FLUTD, which constituted the material of this study, were recorded. Of the 50 animals used in the study, 42% were female and 58% were male. The material consisted of 28% tabby, 22% crossbred and 22% british breed cats. Detailed data were given in Table 1.

Table 1. Breed variable findings of the cats

Race	Frequency	Percentage (%)
Ankara	1	2
British	11	22
Chinchilla	1	2
Sphenks	1	2
Melez	11	22
Persian	3	6
Scottish	6	12
Siyam	2	4
Tabby	14	28
Totally	50	100

In the survival distribution; the rate of animals that died was 14% (n=7), the rate of animals that were followed up was 18% and the rate of animals that recovered was 68%. Of the animals, 30% had struvite crystalluria, 28% had idiopathic cystitis and 20% had signs of renal disease (Table 2). Among the 50 FLUTD cases in our study, partial obstruction was detected in 17 of 29 cases (34%) with idiopathic cystitis (n=5) and struvite crystalluria (n=12) and urine flow was restored by probing in all of these cases.

Table 2. Etiological findings of the cats in the study

Race	Frequency	Percentage (%)
Haemorrhagic cystitis	1	2
Emphysematous cystitis	1	2
Diabetes	1	2
Hydronephrosis	3	6
Idiopathic cystitis	14	28
Constipation	1	2
Pathology (Neoplasia)	1	2
Signs of renal disease	10	20
Presence of sediment in urine	3	6
Struvite crystalluria	15	30
Totally	50	100

According to the data obtained from ultrasonographic evaluation, mean bladder wall thickness (MDK): 0.3590±0.35, ureter width (UG): 0.3484±0.45, kidney width (BEN): 2.6380 ± 0.349, kidney length (BBOY): 3.9024 ± 0.68963, kidney height (BYÜK): 2.5668 ± 0.48963, cortex medulla ratio (C/M): 0.9018±0.44 and renal arterial resistive index (RI): 0.6495±0.09 (Table 3).

Table 3. Mean findings of ultrasonographic values

	M (Mean)	SS (Standard deviation)	Min.	Max.
MDK (cm)	0.3590	0.35842	0.10	2.46
UG (cm)	0.3484	0.45150	0.05	2.38
BEN (cm)	2.6380	0.34930	2.00	3.57
BBOY (cm)	3.9024	0.68963	2.08	3.67
BYÜK (cm)	2.5668	0.48963	1.62	2.24
C/M (cm)	0.9018	0.44409	0.19	3.15
RI	0.6495	0.09050	0.42	0.80

MDK; bladder wall thickness, UG; ureter width, BEN; kidney width, BBOY; kidney length, BYÜK; kidney height, C/M; cortex/medulla ratio, RI; arterial renal resistive index.

According to the mean values of the measured biochemical parameters; BUN: 48.1106±40.95, CREA: 2.2334±3.06, P: 6.2445±2.97, K: 4.1815±0.68, Na: 149.1538±9.01 (Table 4).

Table 4. Descriptive statistics for biochemical parameters

	M (Mean)	SS (Standard deviation)	Min.	Max.
BUN (mg/dL)	48.1106	40.95330	8.75	140.00
CREA (mg/dL)	2.2334	3.06405	0.40	19.19
P (mg/dL)	6.2445	2.97135	0.55	15.00
K (mg/dL)	4.1815	0.68962	3.20	5.50
Na (mg/dL)	149.1538	9.01326	132.00	165.00

BUN; blood urea-nitrogen, Crea; creatinine, P; phosphorus, K; potassium, Na; sodium.

The mean values of haemogram parameters were WBC: 12.4238±8.32, RBC: 9.1592±2.66, HCT 35.0780±10.24 and PLT 243.8000±123.73. The mean values of some urine parameters were PRO: 1.6286±1.03, BIL: 1.9429±0.90, NIT: 0.3429±0.59, DANS: 1000.5143±174.07, pH 6.6541±0.63.

In the evaluation made within this framework, a positive moderate significant correlation was found between RI and P value, while a positive moderate significant correlation was found between RI and BUN and CREA values. Again, a significant correlation close to positive moderate level was found between BEN and Na values. A significant correlation close to positive moderate level was determined between BBOY and P values (Table 5).

Table 5. Correlation analysis results for the relationship between ultrasound values and biochemistry parameters

Ultrasound values	Biochemistry values				
	BUN (mg/dL)	CREA (mg/dL)	P (mg/dL)	K (mg/dL)	Na (mg/dL)
MDK (cm)	-0.138	-0.134	-0.151	-0.132	0.034
UG (cm)	-0.207	-0.148	-0.229	-0.179	-0.242
BEN (cm)	0.140	0.206	0.045	0.039	0.365*
BBOY (cm)	-0.014	0.148	0.338*	0.189	-0.172
BYÜK (cm)	0.031	0.229	0.147	0.084	0.181
C/M (cm)	-0.034	-0.163	-0.072	0.155	0.198
RI	0.385*	0.379*	0.491**	0.277	0.044

*MDK; bladder wall thickness, UG; ureter width, BEN; kidney width, BBOY; kidney length, BYÜK; kidney height, C/M; cortex/medulla ratio, RI; arterial renal resistive index, BUN; blood urea-nitrogen, Crea; creatinine, P; phosphorus, K; potassium, Na; sodium. *p<0.05; **p<0.01*

According to the results of Pearson correlation analysis, a significant relationship between RI and TIT PRO values was found to be positive and close to medium level. Again, a positive medium level significant relationship was found between BBOY and NIT values. There is no significant relationship between other values (Table 6).

Table 6. Correlation analysis results for the relationship between ultrasound values and urine parameters.

Ultrasound values	Urine parameters				
	PRO (mg/dL)	BLO (urine)	NIT (urine)	DANS (urine)	pH (urine)
MDK (cm)	-0.246	0.168	-0.108	0.113	0.171
UG (cm)	-0.277	0.040	0.064	0.114	0.056
BEN (cm)	0.127	-0.065	-0.076	0.293	0.144
BBOY (cm)	0.007	0.108	0.549**	0.043	0.148
BYÜK (cm)	0.044	0.124	0.218	0.097	0.161
C/M (cm)	-0.052	-0.305	-0.011	0.056	0.220
RI	0.396*	0.037	0.178	0.103	0.078

*MDK; bladder wall thickness, UG; ureter width, BEN; kidney width, BBOY; kidney length, BYÜK; kidney height, C/M; cortex/medulla ratio, RI; arterial renal resistive index, PRO; protein, BIL; bilirubin, NIT; nitrite, DANS; density. *p<0.05; **p<0.01*

According to the results of Pearson correlation analysis, a positive strong significant relationship was found between PRO value and BUN and P values. A positive moderate significant correlation was found between PRO and CREA and K values (Table 7).

Table 7. Correlation evaluation results for the relationship between biochemistry values and urine parameters

	PRO (mg/dL)	BIL (mg/dL)	NIT (urine)	DANS (urine)	pH (urine)
BUN (mg/dL)	0.729**	0.149	-0.062	-0.008	-0.150
CREA (mg/dL)	0.557**	0.135	-0.058	0.004	-0.187
P (mg/dL)	0.662**	0.195	0.130	0.049	-0.083
K (mg/dL)	0.519**	-0.024	0.168	-0.290	-0.136
Na (mg/dL)	-0.066	-0.072	-0.206	-0.088	-0.124

*BUN; blood urea-nitrogen, Crea; creatinine, P; phosphorus, K; potassium, Na; sodium, PRO; protein, BIL; bilirubin, NIT; nitrite, DANS; density.*p<0.05; **p<0.01*

When BUN and CREA parameters were compared with survival status, a positive and highly significant relationship was found. The relationship between P, urine PRO parameters and survival status is significant at positive and strong level. The relationship between WBC, K and urine DANS parameters and survival was found to be significant at positive and strong level (Table 8).

Table 8. Comparison of some parameters according to survival status of cats.

Parameters	Groups	Mean	SS(Standart deviation)	P
BUN (mg/dL)	Dead	105.54	43.17	0.000
	Monitoring	53.73	39.01	
	Recovering	34.79	29.85	
CREA (mg/dL)	Dead	6.07	6.06	0.000
	Monitoring	2.46	2.60	
	Recovering	1.38	1.30	
P (mg/dL)	Dead	9.62	3.88	0.003
	Monitoring	5.90	1.59	
	Recovering	5.53	2.47	
K (mg/dL)	Dead	4.81	0.64	0.010
	Monitoring	4.48	0.66	
	Recovering	3.97	0.61	
WBC(10^9/L)	Dead	20.12	11.26	0.019
	Monitoring	13.09	6.23	
	Recovering	10.65	7.37	
PRO (urine)	Dead	2.60	1.14	0.009
	Monitoring	2.16	1.16	
	Recovering	1.29	0.80	
DANS (urine)	Dead	819.00	457.30	0.036
	Monitoring	1023.33	7.08	
	Recovering	1032.62	6.18	

*BUN; blood urea-nitrogen, Crea; creatinine, P; phosphorus, K; potassium, Na; sodium, PRO; protein, BIL; bilirubin, NIT; nitrite, DANS; density.*p<0.05; **p<0.01*

BUN, CREA, P and RI values measured in the 50 cats which were diagnosed as partial obstruction (n=17) and then died (n=7) are presented in the table below (Table 9).

Table 9. Descriptive statistics in cats with partial obstruction (Mean±SE).

Status	CREA (mg/dl)	BUN (mg/dl)	P (mg/dl)	RI
Partial obstruction	1.258±0.311	36.472±7.609	5.341±0.493	0.635±0.021
Dead	6.077±2.293	105.542±16.317	9.628±1.467	0.701±0.045

BUN; blood urea-nitrogen, Crea; creatinine, P; phosphorus, K; RI; resistive index.*p<0.05; **p<0.01

DISCUSSION

Lower urinary tract diseases are frequently encountered in the clinic. The prevalence of lower urinary tract diseases in cats has been reported as 3.3%. The recurrence rate within one year is 1%. It was determined that 4.5%-8% of cats admitted to animal hospitals and veterinary clinics had FLUTD (Lekcharoensuk et al., 2001; Longstaff et al., 2017). In the present study, besides biochemistry and urine analysis data, imaging (ultrasonography) was also used to evaluate the disease. The results contain relative data for patient prognosis.

In a prevalence-based study on the relationship between lower urinary tract diseases and gender in cats, it was reported that the disease was most commonly observed in neutered males, followed by neutered females, active males and active females, respectively (Lekcharoensuk et al., 2001). In another study, it was reported that 82.1% of 185 cats diagnosed with FLUTD were male and 17.9% were female (Nururrozi et al., 2020). In a report, of 119 cats diagnosed with FLUTD; 73.9% were male and 26.1% were female (Savik et al., 2011). In all studies,

it is observed that the risk of FLUTD is higher in males. In this study, 58% of the patients admitted to the clinic were male cats. The data of the study are compatible with other gender studies. The higher incidence of obstruction in male cats may be related to the anatomy of the urethra and the fact that obstructive cats are more easily recognised by the owners than others.

In this study, tabby cats were found to be the most common breed with FLUTD with a rate of 28%. The second most common breed was British and crossbred cats with the same rate of 22%. The third most common breed with FLUTD was Scottish (12%). The other breeds were Persian (6%), Siamese (4%), Angora (2%), Sphenx (2%) and Chinchilla (2%). In a study on obstructive lower urinary tract diseases conducted in Aydın province of Turkey, the breed variability was reported as; Mixed breed 48% (13), Persian 14.81% (4), British 11.11% (3), Scottish 11.11% (3), Siamese 11.11% (3), Ankara 3.70% (1) (Ay et al., 2021). In another study, the breed prevalence in 78 cats diagnosed with FLUTD was determined as Domestic Shorthair (66.7%), Persian (26.9%), American Shorthair (2.6%), Exotic Shorthair, Scottish Fold and Siamese (1.3%), respectively (Piyarungsri et al., 2020). Regional ownership preferences and the fact that the preference for ownership is probably concentrated on some breeds may have been effective on the results of this research.

In this study, in 50 cats with FLUTD, mortality was 14% (n=7) and recovery rate was 68% after one month follow-up. The rate of cats requiring follow-up was 18% (n=9). Savik et al. (2011) determined the mortality rate in cats with FLUTD as 20% (n=10/50), reported that 23 cats (46%) had no recurrence and three cats (6%) were euthanised after diagnosis. In another study, mortality and recurrences due to lower urinary tract obstruction in cats were investigated. Accordingly, mortality was determined as 8.5% and recurrences were 22% in the first six months (Segev et al., 2011).

The most important complications observed in cats with FLUTD are renal problems due to hydronephrosis and acute kidney injury. This condition is frequently encountered in obstructive FLUTD cases (Neri et al., 2016). In the present study, mortality (14%) was thought to be due to renal-related complications and evidence of renal disease (n=10 and CREA; 2.233±3.064 mg/dL, BUN; 48.110±40.953), as well as the relatively high mean age of the cats in the study (6.322±4.412 (Mean±SD) mean age).

Nururrozi et al. (2020) found idiopathic cystitis in 103, urinary tract infection in 47, urolithiasis in 24, urethral plaque in 9, and neoplasia in 1 of 185 cats diagnosed with FLUTD (Nururrozi et al., 2020). In another study, 59 (53%) cats with FLUTD had idiopathic cystitis, while urolithiasis was reported in 13 (12%) cats (Lund et al., 2013). In a study on lower urinary tract infections in cats, crystal-induced obstruction was reported in 21% and bacterial urinary tract infection in 2% of the cats included in the study (Kruger et al., 1991).

In a recent study on 77 cats with FLUTD, 57.7% had idiopathic cystitis and 18% had struvite crystalluria (Piyarungsri et al., 2020). In our study, the most common stone type was struvite 30% (15%). Idiopathic cystitis and renal disease were detected in 28% (n=14) and 20% (n=10) of the cases, respectively. These were followed by hydronephrosis, sediment, haemorrhagic cystitis, emphysematous cystitis and other pathologies (neoplasia). Similar to previous studies (Buffington et al., 1997, Piyarungsri et al., 2020), struvite calculi and idiopathic cystitis were found at high rates in this study.

The presence of uroliths may vary according to the diet of cats (Osborne et al., 2009). In a study of 131 cats, 44.3% struvite stones and 43.5% calcium oxalate stones were found (Ortega et al., 2023). The results of another 25-year study showed that the rate of urinary stones encountered in cats in 1981 was 78% struvite and 2% calcium oxalate, while this rate increased to 55% for calcium oxalate and decreased to 33% for struvite between 1994-2002. In 2006, the incidence of struvite increased again and reached 50%, while calcium oxalate was found to be 39% (Osborne et al., 2009).

Although FLUTD is a disease of the lower urinary tract, it may be associated with different diseases. If FLUTD progresses and/or treatment is started late, renal failure may occur (Guun-Moore, 2003). FLUTD may be accompanied by other systemic diseases such as diabetes mellitus. Results of a study in 141 cats with diabetes mellitus showed that 18 cats also had urinary tract infections (Bailiff et al. 2006). In another study investigating urinary tract infections in cats with hyperthyroidism, diabetes and chronic kidney disease, it was reported that urinary tract infections were observed in 12% of 90 cats with hypertroidism, 12% of 57 cats with diabetes and 22% of 77 cats with chronic kidney disease (Mayer-Roenne et al., 2007). In another study on chronic renal failure, bacterial urinary tract infection was determined in 16.7% of 74 cats (Hostutler et al., 2005). In the present study, the 'n' number was lower than in the aforementioned studies, and 10 of the 50 cats with FLUTD had evidence of renal diseases, only one cat had emphysematous cystitis and one cat had diabetes mellitus. These results also indicate that FLUTD may present clinically in different ways.

FLUTD is affected by systemic diseases and renal pathologies. Therefore, blood parameters may vary in each FLUTD patient (Guun-Moore, 2003). In a study, HCT 35.26 ± 3.82 and WBC 17.436 ± 7.757 were determined in cats with obstructive FLUTD, while HCT 34.65 ± 4.73 and WBC 6.088 ± 2.728 were determined in cats with nonobstructive FLUTD (Evangelista et al., 2023). In the present study, WBC was 12.4238± 8.32 and HCT was 35.0780± 10.24.

WBC and HCT are affected by systemic diseases, secondary infections and conditions such as chronic renal failure (Wood, 2017). The results of the present study are similar to the results mentioned above.

In a study, serum biochemistry values were checked in cats with urethral obstruction. It was reported that 28 of the cats constituting the material were nonazotemic, 19 cats had moderate azotemia, and 35 cats had severe azotemia. It was also reported that 33 of these cats had severe hyperkalaemia (Nevins et al., 2015). In another study, urea was determined above the reference value in 21 of 26 cats with obstructive FLUTD. Creatinine was determined above the reference range in 20 cats (Neri et al., 2016). In our study, the mean BUN value of the cats was 48.11±40.95 and the mean CREA value was 2.23±3.06. This result is compatible with the studies of Nevins et al. (2015) and Neri et al. (2016). Among the 50 FLUTD cases in our study, 'partial obstruction' was detected in 17 (34%) of a total of 29 cases of idiopathic cystitis and struvite crystalluria. In all of these patients, the obstruction was opened by probing. Of these patients, 5 were found to have idiopathic cystitis and 12 had struvite crystalluria. The mean BUN, CREA and P values measured in these cases (n=17, partial obstruction) were 36.4718±7.6099, 1.2588±0.3109 and 5.3407±0.4928, respectively (mean±SE). Urethral obstruction can be seen at a high rate of 18-58% in FLUTD cases. FLUTD can also be classified as obstructive and non-obstructive (Ay et al., 2021; Sabino, 2017; Sevik et al., 2011 and Woolf, 2012). In obstructive FLUTD cases, the kidneys are affected due to the disruption of urine flow. Therefore, an increase in BUN and CREA levels is observed. RI values measured in FLUTD cases in which the kidney is affected also increase (Evangelista et al., 2023). In the present study, a significant positive correlation was found between RI and BUN and CREA values in the evaluation of 50 cats (partially obstructive and non-obstructive). The RI value measured for all cases was below the reference range of 0.7, and when evaluated separately; 0.6350±0.021 (mean±SE) in cases of partial obstruction (n=17) and 0.7014±0.045 (mean±SE) in dead cats (n=7), which were higher in this group, were determined in the measurements made at the first presentation to the clinic in all the cats.

Mortality in renal diseases is associated with kidney-related complications, tumour presence and geriatrics (Bartges et al., 1996; Segev et al., 2011; Sabino, 2017; Webb, 2018). In this study, when BUN and CREA levels were compared with survival status, it was revealed that there was a positive very strong significant relationship (0.05<p).

Urine analysis is an easy, fast and practical method for the diagnosis and prognosis of FLUTD. Microscopic examination of urine samples is a necessary application in diagnosis (Lulich 2007). In this study, struvite crystalluria was found in 15 of 50 cases and the presence of inflammatory sediment was found in three cases. On the other hand, PRO 1.63±1.03, BIL 1.94±0.90, NIT 0.34±0.59, DANS 1000.5±17.41 and pH 6.65±0.63 were determined in complete urine analysis (TIT).

Proteinuria in cats is usually associated with conditions such as urinary tract infections, high-protein diet feeding, urinary tract-related pathologies, hypertension, diabetes, parasites and acute/chronic renal failure (Wood, 2017). Evangelista et al. (2023) in their study, according to TIT analysis data in 21 cats with obstructive FLUTD; determined the protein level as +3 and +2 in non-obstructive cats. Obstructive FLUTD is the most common and fatal cause of postrenal acute renal injury. Acute renal injury results from increased pressure within the renal pelvis and ureter reducing renal blood flow and glomerular filtration rate. Loss of renal function was determined within 24 hours after the obstructive event and acute kidney injury was detected. Azotemia and hyperkalemia are mainly encountered in acute renal damage (Fischer et al. 2009). In this thesis study, serum potassium concentration was measured as 4.18±0.68, while proteinuria in TIT was on average; It was detected in the +1, +2 range. Hyperkalaemia is an early sign of acute urinary obstruction. It is not surprising to see hyperkalaemia in obstructive FLUTD cases. In the study, a positive moderate significant correlation was found between TIT PRO and CREA and serum potassium values. In addition, a positive moderate significant correlation was found between TIT PRO and measured RI values. According to the results of Pearson correlation analysis, a positive strong significant correlation was found between TIT PRO value and BUN and P values (0.05<p). In this study, the relationship between WBC, potassium and TIT DANS parameters and survival was found to be strongly positive and significant (0.05<p).

In veterinary medicine, urine density can be measured by complete urinalysis (TIT) or refractometry. In the study, densitometry was evaluated by TIT. In healthy cats, an average concentrated urine of 1.035 and above is produced. Many factors can cause high or low density. Apart from the kidney, endocrine diseases, steroid use, diuretic drugs, fluid therapy or organic solutes such as protein and amino acids affect the density (Wood, 2017). In a study performed in 21 obstructive cats, the mean pH was found to be 6.6 ± 0.7 and the density was found to be 1.025.

In cats with non-obstructive FLUTD, the mean pH was determined as 7.8 ± 1.5 and the density was determined as 1.040 (Evangelista et al., 2023). TIT results in cats with FLUTD experiencing short- and long-term obstruction were reported as follows: In short-term obstruction; pH was 7.92±0.12, in long-term obstruction 8.25±0.35 and the density was 1.022.36±3.27 in short-term obstruction and 1018.75±6.46 in long-term obstruction. The amount of TIT protein was determined as +2 and +3 in both groups, respectively (Ay et al. 2021). In the presented

study, it was evaluated that the underlying factors causing FLUTD, although they did not lead to severe renal failure, could affect renal function acutely.

In this study, the pH value was determined as 6.65±0.63. pH in cats is affected by urinary tract infections and bacteria causing infection can increase urine pH (Rizzi 2014). The urine pH of cats diagnosed with struvite crystalluria is usually high, that is, alkaline (≥6.6). On the other hand, calcium oxalate crystalluria is more common in male cats, which usually have acidic urine pH (Grauer 2015). It has been observed that the urine pH of cats fed in their natural environment varies between 6.0 and 7.0 (Timothy 1996). Similar results were obtained in this study, and reasons such as feeding with commercial dry food, inactivity, drinking little water, and the cat being a male are all causes of struvite crystalluria (Kelliher 2022).

Morphological evaluation of kidney and urinary bladder is important in patients with FLUTD. Measurements contribute to the diagnosis of the disease. It has been explained that kidney size may vary according to race, previous disease, and gender in ultrasonographic evaluation (Debruyn et al. 2012). In our study; mean MDK was determined as 0.36±0.35, UG as 0.35±0.45, BEN as 2.638±0.349, BBOY as 3.902±0.689, BYUK as 2.567±0.489, C/M as 0.90±0.44, RI as 0.65±0.09. In one study, kidney thickness (cm) was determined as 0.26 ± 0.11 in cats with non-obstructive FLUTD and 0.19 ± 0.06 in cats with obstructive FLUTD. Kidney length (cm) in the left kidney was 3.87 ± 0.24 in non-obstructive and 4.49 ± 0.43 in obstructive. In a study on obstructive and non-obstructive FLUTD, urinary bladder wall measurements (cm) were measured as 0.12 ± 0.05 in the control group, 0.26 ± 0.11 in the non-obstructive group and 0.19 ± 0.06 in the obstructive group. Renal pelvis (cm) was found to be 0.09 ± 0.02 in healthy cats, 0.08 ± 0.03 in non-obstructive cats, and 0.28 ± 0.16 in obstructive cats (Evangelista et al., 2023). In our study, kidney measurements of obstructive and non-obstructive cats were determined to be within the normal range. According to a study, kidney length was calculated as 3.93 ± 0.23 in the right kidney and 3.87 ± 0.24 in the left kidney in cats with non-obstructive feline lower urinary tract disease (n=8), and 4.51 ± 0.47 in the right kidney and 4.49 ± 0.43 in the left kidney in cats with obstructive feline lower urinary tract disease (n=11) (Evangelista et al. 2023). In our study; a renal thickness value similar to the study conducted by Evangelista et al. (2023) was found. However, this value was determined as an average of 0.19 in obstructive patients, and our value is lower compared to the results of the aforementioned study. This situation may be related to the partial obstruction detected in 17 of the 50 cats constituting the material in this presented study, the absence of complete obstructive cases in the material, and the continuation of urine output, albeit intermittently (in the anamnesis), in these semi-obstructive patients. Again, in our study, the urinary bladder wall thickness was found to be higher compared to the results of the study conducted by Evangelista et al. (2023). Struvite crystalluria (30%), idiopathic cystitis (28%), emphysematous cystitis (2%) and hemorrhagic cystitis (2) detected in the cases constituting the material in our study are factors that generally increase the urinary bladder wall several times (Lemberger et al. 2011). Renal resistive index (RI); It is the sonographic doppler index of intrarenal arteries, also defined as (peak systolic velocity - end-diastolic velocity) / peak systolic velocity. Calculation is made after obtaining images with Pulsed Wave Doppler (PW Doppler). Renal resistive index is a non-specific prognostic marker in vascular diseases affecting the kidney. It can be used in the evaluation of renal function (Tipisca et al. 2015). In a study conducted, RI measurement was evaluated in healthy cats. The mean left kidney RI value in 31 cats included in the study was found to be 0.61±0.05 (Ostrowska et al. 2016). A general mean of up to 0.7 can be considered healthy (Novellas et al. 2007). In another study conducted on 116 cats, the mean RI value was determined as 0.59 ± 0.08 in 24 healthy cats, while it was measured as 0.73 ± 0.12 in chronic renal failure cases and 0.72 ± 0.08 in acute renal failure cases in 92 sick cats. Gender and age did not affect RI (Tipisca et al. 2015). In another study conducted on cats with FLUTD, the RI value was determined as 0.65 (0.54–0.68) in the measurements performed on the left kidney in the control group, while it was determined as 0.73 (0.61–0.75) in non-obstructive FLUTD cases and 0.69 (0.54–0.93) in obstructive FLUTD cases. Regardless of obstructive or non-obstructive FLUTD, chronic or acute renal failure accompanying the disease definitely leads to an increase in the renal resistive index (Evangelista et al., 2023).

In this thesis study, the mean RI value in the left kidney was measured as 0.65±0.09. When compared with other studies, it was evaluated that the normal RI values measured in this study could be related to reasons such as the partial obstruction observed in the animals forming the material, the cases being uncomplicated and not having developed severe renal damage. It is a fact that renal blood flow changes in cats with FLUTD need to be investigated in detail.

CONCLUSSION

The results of this study emphasize that FLUTD cases are mostly seen in male cats (58%) and that there are significant differences between breeds. While the mortality rate in 50 FLUTD cats was determined as 14% (n=7), the recovery rate was determined as 68%. The most common problems in the study were struvite crystalluria (30%) and idiopathic cystitis (28%), respectively. The mean serum BUN and CREA concentrations measured in

cats with FLUTD were found to be relatively high, although there were individual differences. However, it is noteworthy that in cases where partial obstruction was observed among 50 FLUTD cases (n=17), neither values increased in terms of mean value, although there were individual differences. Hemogram değerleri de ortalama olarak normal sınırlar arasında belirlenmiştir. Hemogram values were also determined to be within normal limits on average. It was determined that BUN, CREA and P values measured in cats determined to be dead during follow-up were higher than in surviving animals. It is noteworthy that the average density value determined in TIT was low. A positive significant relationship was determined between urine protein and serum BUN and P values. Renal measurement data evaluated in this study were recorded within average normal values, although there were some individual differences between the cases. The resistive index value measured in the study was below the normal upper value of 0.7. It is noteworthy that blood urea-nitrogen and creatinine values were high in cases with partial obstruction in this study. After a one-month follow-up, it was determined that in cats that were determined to have died, blood urea-nitrogen, creatinine and phosphorus values measured at the first application to the clinic were higher than in surviving animals. The results emphasize the importance of comparative evaluation of all measurement data in the diagnosis, patient follow-up, determination of prognosis and determination of the correct treatment method in cases of cat lower urinary tract disease. In these cases, special medical treatment applications and diets can be preferred according to the type of cat lower urinary tract disease and the underlying primary cause, and these may prevent possible recurrences. Additional studies with increased material numbers should be conducted in order to reveal the relationship between the parameters in more detail.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: EE and TC contributed to the project idea, design and execution of the study. EE and TC contributed to obtaining the data. TC and EE analyzed the data. EE and TC drafted and wrote the article. EE and TC critically reviewed the manuscript. All authors read and approved the final manuscript.

Ethical approval: For the research, Afyon Kocatepe University Animal Experiments Local Ethics Committee (AKÜ-HADYEK) was applied and necessary permissions (04.10.2022 date and 99 number) were obtained.

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RESEARCH ARTICLE

Evaluation of Some Spermatological Parameters Following *Escherichia coli* Contamination in Bull Semen

Burcu ESİN[1]*, Merve Gizem SEZENER KABAY[2], Cumali KAYA[1], Volkan Enes ERGÜDEN[2]

[1]*Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Samsun, Türkiye*
[2]*Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Samsun, Türkiye*

ABSTRACT

This study aimed to evaluate the effects of extended-spectrum beta-lactamase (ESBL) producing and non-producing Escherichia coli concentrations on bull semen motility parameters and spermatozoa viability. A total of 50 frozen semen straws from the same Simmental bull were used. All semen straws were thawed in a water bath at 37 °C and divided into 5 groups of 10 semen samples. Group 1 (G1) (n=10) was the uncontaminated control group. Group 2 (G2) (n=10) was contaminated with 100.000 cfu/ml and Group 3 (G3) (n=10) was contaminated with E. coli ATCC 25922 at a concentration of 1.000.000 cfu/ml. Group 4 (G4) (n=10) was contaminated with 100.000 cfu/ml and Group 5 (G5) (n=10) was contaminated with ESBL producing E. coli BAA-196 at a concentration of 1.000.000 cfu/ml. In the study, progressive motility, motility and percentages of dead-live spermatozoa in semen samples of these groups were analyzed over time. Significant decreases in spermatozoa motility and viability were observed and the most significant effects were seen in groups (G4 and G5) contaminated with high concentrations of ESBL-producing E. coli ($p<0.05$). It was determined that the effect of bacterial contamination on spermatological parameters was dose-dependent, with higher concentrations causing more rapid and severe deterioration in semen quality. These findings highlight the role of bacterial contamination, especially with resistant strains, in reducing semen quality and draw attention to the importance of microbial contamination in artificial insemination practices. Further research is needed to explore alternative methods to control contamination in reproductive technologies and combat antibiotic resistance.

Keywords: Bull, Contamination, *Escherichia coli*, Sperm parameters

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Boğa Spermasında Escherichia coli Kontaminasyonu Sonrası Bazı Spermatolojik Parametrelerin Değerlendirilmesi

ÖZ

Bu çalışma, genişlemiş spektrumlu beta laktamaz (ESBL) üreten ve üretmeyen Escherichia coli konsantrasyonlarının boğa sperma motilite parametreleri ve spermatozoa canlılığı üzerindeki etkilerini değerlendirmeyi amaçlamıştır. Aynı Simental boğasından toplam 50 adet donmuş sperma payeti kullanıldı. Tüm sperma payetleri 37 °C'de su banyosunda çözdürülerek, her biri 10 sperma örneğinden oluşan 5 gruba ayrıldı. Grup 1 (G1) (n=10), kontamine edilmeyen kontrol grubunu oluşturdu. Grup 2 (G2) (n=10), 100,000 cfu/ml ve Grup 3 (G3) (n=10), 1,000,000 cfu/ml konsantrasyonunda E. coli ATCC 25922 ile, Grup 4 (G4) (n=10), 100,000 cfu/ml konsantrasyonda ve Grup 5 (G5) (n=10), 1,000,000 cfu/ml konsantrasyonda ESBL üreten E. coli BAA-196 ile kontamine edilen grupları oluşturuldu. Çalışmada bu gruplara ait sperma örneklerinde progresif motilite, motilite ve ölü-canlı spermatozoa yüzdelerinin zaman içinde değişimi analiz edildi. Spermatozoa motilitesi ve canlılığında önemli azalmalar gözlemlendi ve en belirgin etkiler, yüksek ESBL üreten E. coli konsantrasyonlarıyla kontamine olan gruplarda (G4 ve G5) görüldü ($p<0.05$). Bakteriyel kontaminasyonun sperma parametreleri üzerindeki etkisinin doza bağlı olduğu ve daha yüksek konsantrasyonların sperma kalitesinde daha hızlı ve ciddi bozulmaya neden olduğu belirlendi. Bu bulgular, özellikle dirençli suşlarla bakteriyel kontaminasyonun sperma kalitesini azaltmadaki rolünü vurgulamakta ve suni tohumlama uygulamalarında mikrobiyal kontaminasyonun önemine dikkat çekmektedir. Reprodüktif teknolojilerde kontaminasyonu kontrol etmede ve antibiyotik direnciyle mücadele etmeyi sağlamada alternatif yöntemleri keşfetmek için daha fazla araştırmaya ihtiyaç vardır.

Anahtar kelimeler: Boğa, *Escherichia coli*, Kontaminasyon, Sperm parametreleri

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ORCID ID; *BE: 0000-0002-5728-1478, MGSK: 0000-0003-0487-7515, CK: 0000-0002-0666-5359, VEE: 0000-0003-2215-2868*
*Corresponding author e-mail: burcuyalcin@omu.edu.tr

INTRODUCTION

In cattle breeding, various genetic diseases or environmental factors are associated with infertility. In addition, bacterial contamination of cattle semen significantly contributes to reduced birth rates and increased prevalence of reproductive disorders in cattle (Thibier and Guerin 2000). The known mechanisms by which infection causes infertility include (a) bacterial adhesion to spermatozoa, (b) immobilizing factors produced by certain bacteria, (c) enhanced activation of the immune system, and (d) disruption of glandular function (Cottell et al. 1996).

The presence of bacteria in ejaculates may originate from intrinsic bacterioses within the male urogenital system, extending from the testes to the penis and prepuce (Marcus et al. 1994). Commonplace bacteria can also be introduced through the artificial vagina, lab equipment, semen extenders, or even the lab environment, even in the face of stringent biosecurity regulations during semen collection. (Rana et al. 2012). Additionally, the presence of bacterial pathogens such as *Staphylococcus* spp*., Micrococcus* spp*., Escherichia coli (*E. *coli), Pseudomonas* spp*., Corynebacterium* spp*., Proteus* spp*., Klebsiella* spp*.,* and *Bacillus* spp. has been reported in frozen semen (Mitra et al. 2016). Bacteria such as *E. coli* significantly reduce sperm motility and increase sperm agglutination. *E. coli* has been shown to adhere to the sperm membrane/surface through mannose-binding sites (Monga and Roberts 1994; Wolff et al. 1993). Class A β-lactamases, also known as extended-spectrum β-lactamases (ESBLs), are a fast developing category of β-lactamases that may hydrolyze and produce resistance to monobactams (aztreonam) and oxy-imino cephalosporins (cefotaxime, ceftazidime, ceftriaxone, cefuroxime, and cefepime) (Peirano and Pitout 2010). Calves exhibited the highest prevalence of ESBL-producing *E. coli* on mixed farms, with %56.2 of fecal samples testing positive for this pathogen. This was followed by cows, with %41.1 of fecal samples, and beef cattle, which showed a prevalence of %21.4 in their fecal samples (Schmid et al., 2013). Semen samples containing these bacteria have been associated with reproductive failure following insemination. Pathogenic bacterial contamination of ejaculates can impair spermatological parameters by reducing sperm motility and viability, causing morphological abnormalities, and decreasing sperm concentration (Folliero et al. 2022). Moreover, antibiotic-resistant *E. coli* strains can exert more rapid and severe adverse effects on sperm function. These resistant bacteria produce toxins and metabolites that disrupt sperm motility, viability, and morphological integrity. Such bacterial infections can damage sperm membrane structure and compromise DNA integrity, reducing viability and motility. Additionally, they may increase oxidative stress, resulting in cellular damage (Oghbaei et al. 2020). These spermatological parameters play a crucial role in fertility (Khalili et al. 2000). Although bacterial contamination of ejaculates contributes to infertility in cattle, standardized protocols for the routine microbiological analysis of bovine semen remain lacking. Additionally, the microbial flora of semen can also induce infertility in females, increase embryonic mortality rates, reduce pregnancy success, and, on a larger scale, lead to abortion and other reproductive complications. In addition, failure to control contamination in reproductive biotechnologies can lead to negative outcomes such as infectious infertility, endometritis and abortion in the cows, and can seriously reduce fertilization success rates by reducing the quality of the semen used (Stringfellow et al. 2000). Given the widespread use of artificial insemination in livestock production and genetic improvement, addressing bacterial contamination in semen is essential (Garba et al. 2023). Therefore, the identification of opportunistic bacteria and the prevention of potential transmission between bulls and from bulls to cows is crucial, as it may help prevent economic losses in cattle production. Furthermore, future bacteriospermia therapy and prevention may benefit from an understanding of the mechanisms behind bacterial damage in spermatozoa (Cojkic et al. 2021; Ďuračka, et al. 2021).

This study aims to investigate the effects of *E. coli*, the most common bacterium found in feces and associated with contamination risk during semen collection, on various spermatological parameters in commercially available semen used routinely by veterinarians in artificial insemination practices.

MATERIALS and METHODS

The study was conducted in the laboratories of the Department of Reproduction and Artificial Insemination and the Department of Microbiology at the Faculty of Veterinary Medicine, X University. The material used in the study consisted of 50 randomly selected semen straws, each with a volume of 0.25 ml, from imported bull semen. These straws were all from a single bull, collected on the same date, and all analyses throughout the study were performed on these samples.

Experimental Design and Group Formation

Before the start of the study, three Simmental semen straws were randomly selected from the nitrogen tank and thawed in a 37 °C water bath to assess initial sperm motility and concentration, which were determined to be 70–80% and 10–20 × 10^6 spermatozoa per straw, respectively. The aim was to check for any significant differences between the straws. All materials used were subjected to sterilization procedures, and the temperature was maintained at 37 °C throughout the study.

All straws (n=50) were thawed at 37 °C for 30 seconds and then divided into five experimental groups (n=5x10). Subsequently, the semen samples were contaminated with *E. coli* at varying concentrations and prepared in the microbiology laboratory. Spermatological parameters (motility and live-dead sperm ratio) were evaluated at 20-minute intervals during the first two hours and subsequently at hourly intervals.

Microbiological Process

E. coli ATCC 25922 and ESBL producing *E. coli* BAA-196 were thawed at room temperature from -20 °C and then passaged into 5 ml of Brain Heart Infusion Broth (1.10498, Merck, KGaA, Darmstadt, Germany), where it were incubated at 37 °C for 18 hours. The resulting bacterial culture was serially diluted 10-fold in sterile physiological saline (FTS, pH=7.2). Two aliquots were plated from each dilution onto MacConkey Agar (1.05465, Merck, KGaA, Darmstadt, Germany) and incubated at 37 °C for 18 hours. Colonies were counted, and the bacterial concentrations in the dilutions were determined, considering McFarland standards. The bacterial suspensions, stored at +4 °C in the refrigerator, were added to the semen samples as soon as the bacterial concentrations (cfu/ml) were determined.

In this context, Group 1 (G1) (n=10) consisted of 10 semen samples of 0.25 ml each, thawed at 37 °C in a water bath, forming the control group. The semen samples were incubated in a 37 °C incubator with %5 CO_2 to evaluate spermatological parameters. Group 2 (G2) (n=10) consisted of semen samples thawed using the same method and contaminated with *E. coli* ATCC 25922 at a concentration of 100.000 cfu/ml and were incubated at 37 °C with %5 CO_2. Group 3 (G3) (n=10) involved semen samples thawed using the same method, contaminated with *E. coli* ATCC 25922 at a concentration of 1.000.000 cfu/ml and incubated under the same conditions (37 °C, %5 CO_2). Group 4 (G4) (n=10) involved semen samples thawed using the same method and contaminated with ESBL producing *E. coli* BAA-196 at a concentration of 100.000 cfu/ml and were incubated under the same conditions (37 °C, %5 CO_2). Group 5 (G5) (n=10) consisted of semen samples thawed using the same method, contaminated with ESBL producing *E. coli* BAA-196 at a concentration of 1.000.000 cfu/ml, and incubated under the same conditions (37 °C, %5 CO_2). Spermatological parameters were evaluated for all groups.

Spermatological Analysis

Sperm motility was evaluated using a Computer-Assisted Sperm Analyzer (CASA) system (SCA, Sperm Class Analyzer, Version 6.5.0.91; Microptic, Barcelona, Spain). The samples were examined under a phase-contrast microscope (Nikon, Eclipse, Tokyo, Japan) equipped with a 10× objective lens and a high-speed camera (60 frames/sec) at 37 °C. At least 200 randomly selected spermatozoa were analyzed for each sample from at least five microscopic fields.

Sperm viability was assessed using eosin-nigrosin staining. For this, 15 µL of eosin-nigrosin stain and 10 µL of semen were placed side by side on a glass slide and gently mixed with a pipette tip. A cover slip spread the sample across the slide at a 30° to 40° angle. The slide was placed on a heating plate until dry, then evaluated under a light microscope at 200x magnification (with immersion oil). Spermatozoa that appeared partially or fully pink or red were considered dead, while spermatozoa that did not absorb the stain were considered alive.

Statistical Analysis

Statistical analyses were performed using RStudio (version 2023.06.0+421, "Mountain Hydrangea") (R Core Team, 2023). Data normality was assessed using the Shapiro-Wilk test, and none of the parameters followed a normal distribution ($p<0.05$). Therefore, non-parametric tests were employed for statistical comparisons.

For each of the three general evaluation parameters, differences between the five groups were assessed at each of the ten-time points using the Kruskal-Wallis test. When significant differences were detected, pairwise comparisons were conducted using Dunn's test, with Holm's method applied for p-value adjustment. The significance thresholds were set as follows: $p<0.05$ (significant), $p<0.01$ (highly significant), and $p<0.001$ (very highly significant).

RESULT

This study aimed to evaluate the effects of *E. coli* contamination on the spermatological parameters of bull semen. The data demonstrate that *E. coli* contamination harmed sperm progressive motility, live sperm and motility rates (Figure 1).

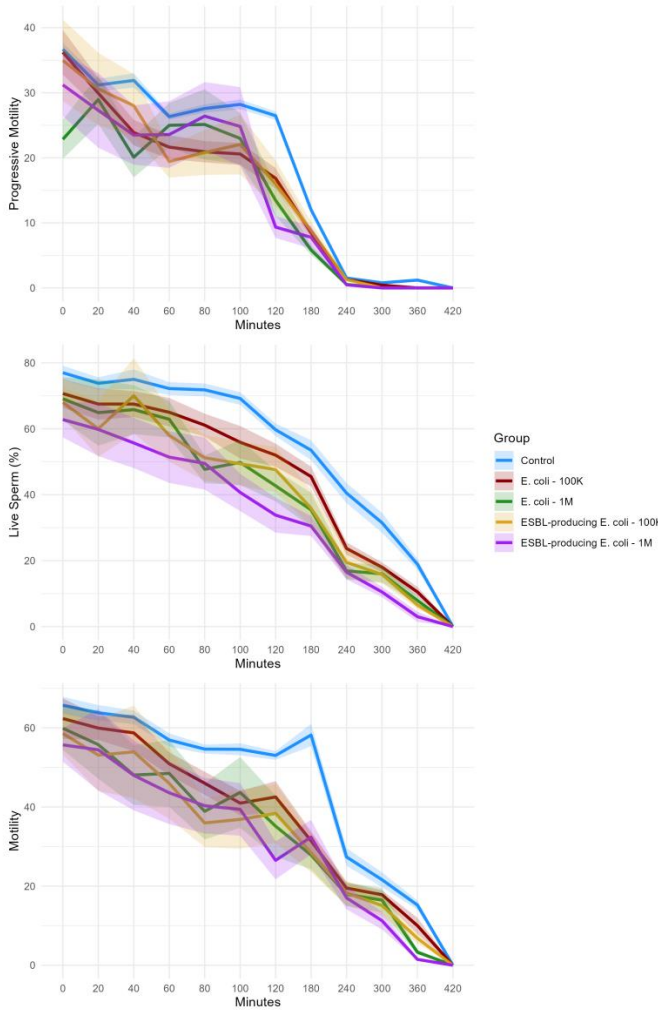

Figure 1. Changes in progressive motility (%), live sperm (%) and motility (%) parameters over time as a result of *E. coli* contamination, respectively. [blue line, control group (G1); pink line, 100.000 cfu/ml *E. coli* contaminated group (G2); green line, 1.000.000 cfu/ml *E. coli* contaminated group (G3); yellow line, 100.000 cfu/ml *E. coli* contaminated group (G4); purple line, 1.000.000 cfu/ml *E. coli* contaminated group (G5)]

Progressive Motility: Group 1 (G1) significantly differed from Groups 2 (G2) and 4 (G4) starting from the 60th minute ($p<0.05$). At the 120th minute, a notable decrease in Group 5 (G5) was observed ($p<0.001$). At the 180th minute, Group 1 (G1) was significantly different from all other groups ($p<0.001$). At the 300th and 360th minutes, differences between Groups 3-5 (G3-G5) disappeared, with the effects of the high-density ESBL-producing groups becoming more pronounced in the later hours ($p<0.01$).

Live Sperm Percentage: At the 100th minute, Group 1 (G1) showed a significant difference compared to Groups 3-5 (G3-G5) ($p<0.01$). At the 180th minute, the difference between Groups 2 and 5 (G2-G5) became statistically significant ($p<0.01$). At the 240th minute, Group 1 (G1) significantly differed from all other groups ($p<0.001$). At the 360th minute, a significant difference was observed between Groups 3 and 5 (G3-G5) ($p<0.05$), with the lowest viability recorded in Group 5 (G5).

Total Motility: Significant differences between Group 1 (G1) and Groups 3-5 (G3-G5) were observed starting from the 80th minute ($p<0.01$). At the 120th minute, Group 5 (G5) exhibited the lowest total motility ($p<0.001$). At the 240th minute, Group 1 (G1) significantly differed from all other groups ($p<0.05$), while at the 360th minute, a significant difference was observed between Groups 4 and 5 (G4-G5) ($p<0.01$).

In particular, sperm samples contaminated with ESBL producing *E. coli* strains showed a more rapid decline in motility over time, significantly reducing sperm viability rates. These findings indicate that resistant bacteria degrade sperm quality more quickly and distinctly, suggesting that such contamination could negatively affect spermatozoon functions. Additionally, it was found that contamination density (100.000 vs. 1.000.000 bacteria/ml) had an even more detrimental effect on spermatological parameters. These results highlight that microbiological contamination of bull semen, especially with resistant bacteria, poses a serious risk to sperm quality preservation and reproductive health.

DISCUSSION

This study evaluated the effects of different concentrations of ESBL-producing and non-producing *E. coli* bacteria on bull sperm motility and viability. Measurements taken at various time points during the study demonstrated the negative effects of bacterial contamination on sperm function. The results showed that *E. coli* had detrimental effects on sperm motility, progressive motility, and viability rates, with the impact being more rapid and pronounced in the presence of resistant bacteria.

Progressive motility data indicated a significant decrease in sperm cells exposed to higher bacterial concentrations. While no significant differences were found between groups at 20 minutes, significant differences were observed from 40 minutes onward, particularly between G1 (control group), G3 (1M *E. coli*), and G5 (10M ESBL-producing *E. coli*) groups. This suggests that contamination intensity negatively impacts sperm motility from the early stages. Notably, at 120 minutes, G2 was not statistically different from G3 and G4, but G2 and G4 significantly differed from G5 (which contained both high-density and ESBL-producing *E. coli*). This implies that contamination intensity has a more substantial effect in the early stages. In the mid-stage (120 minutes), ESBL-producing *E. coli* groups were more affected than non-producing strains, with only the group exposed to high-density and ESBL-producing contamination being statistically distinct from the others. By 300 minutes, the significant differences between G3, G4, and G5 had disappeared, but G2 remained significantly different from these three groups. The delayed negative effect on progressive motility in G2 compared to the other groups may be related to the lower contamination density and the influence of non-ESBL-producing *E. coli*, which was statistically significant. At

360 minutes, G1 was significantly different from all other groups, while no significant differences were observed between G3, G4, and G5, similar to what was observed at 300 minutes. Live sperm percentage data revealed the impact of bacterial exposure on sperm viability. From 60 minutes onward, significant differences between G1 and the G3, G4, and G5 groups became apparent, indicating that sperm viability is highly sensitive to bacterial contamination. Starting from 100 minutes, it was found that G5 had the lowest viability rate, suggesting that high bacterial concentrations may have a toxic effect on sperm cells. At 360 minutes, G1 significantly differed from all other groups, and the differences between G3, G4, and G5 became more pronounced. Total motility results followed a similar trend. Significant differences were observed between G1 and, particularly, G3, G4, and G5 groups at all time points. From 60 minutes onward, the impact of ESBL-producing *E. coli* on motility became more pronounced in the affected groups. From 100 minutes onward, especially at 360 minutes, it was observed that G1 differed from all other groups. This indicates that the effect of bacterial infection on sperm motility progressively increased and became more distinct over time.

Overall, our study confirms the negative effects of ESBL-producing *E. coli* on sperm motility and viability. It was determined that groups containing high concentrations of ESBL-producing *E. coli* exhibited earlier and more pronounced effects than groups with lower concentrations. However, over time, all infected groups showed similar profiles in terms of sperm motility and viability. This suggests that the effects of bacterial contamination on spermatological parameters may reach a saturation point as time progresses.

These results are consistent with previous studies, confirming that bacterial contamination impairs sperm function and leads to motility loss due to metabolic disturbances in sperm cells (Yániz et al. 2010; Kuster and Althouse 2016). The deterioration of spermatological parameters, particularly sperm motility, due to bacterial contamination has also been reported in earlier research. In contaminated sperm samples, bacterial metabolites and toxins harm spermatozoa's cellular structure, leading to motility loss (Diemer et al. 2003; Ďuračka et al. 2021). There are different studies in various species. In a study conducted in boars, it was reported that progressive motility was significantly reduced and abnormal morphology rates increased in ejaculates contaminated with bacteria such as Escherichia coli (Gaczarzewicz et al., 2016). These deteriorations were explained by the deformation of the sperm membrane structure by bacterial endotoxins. Similarly, in a study conducted in boars, it was stated that total and progressive motility were significantly reduced in samples with bacteriospermia, and at the same time, an increase in reactive oxygen species (ROS) was observed (Kuster et al., 2016). This situation shows that bacteria impair sperm functions through oxidative stress. In a study conducted in dogs, a negatively significant correlation was found between sperm motility and colony forming unit (CFU) numbers in samples with high bacterial load in the ejaculate (Sorkytė et al., 2024). Researchers stated that in addition to direct cell membrane damage, bacteria also change environmental parameters such as pH and osmolarity, creating an unfavorable environment for sperm survival.

The mechanisms by which *E. coli* damages sperm cells occur through the endotoxins and various proteases produced by the bacteria. These toxins cause sperm cell membrane disruption, which raises oxidative stress and damages cells (Oghbaei et al. 2020). Additionally, type 1 adhesion molecules, which are present in both bacterial pili and spermatozoa, respectively, and which can be rendered inactive by preincubation with mannose, are used by *E. coli* to attach to the surface structures of spermatozoa. Spermatological parameters decline as a result of these adhesion processes, which alter and harm the plasma membrane and other surface features of spermatozoa (Mayer et al. 2000). A study investigating the prevalence of bacterial contamination of semen and whether contamination can reduce sperm quality has shown that contamination of sperm samples by certain species is more closely associated with infertility. The study showed that E. coli has a generally negative effect on sperm quality in men with infertility (Moretti et al. 2009). The findings of present study indicate that resistant bacteria accelerate these mechanisms, causing sperm quality to degrade more rapidly. This is an important finding, suggesting that the deterioration of spermatological parameters is not only related to the increase in bacterial count but also to the resistance characteristics of the bacteria.

The effect of contamination density on sperm motility was also noteworthy in our study. Samples contaminated with 1.000.000 *E. coli* cells/ml exhibited a more pronounced decrease in sperm motility, whereas samples with 100.000 *E. coli* cells/ml showed a more limited change. These results indicate that the effects of contamination density on sperm function are directly proportional, meaning that as bacterial density increases, sperm quality rapidly deteriorates.

CONCLUSION

The data from this study provide important insights for clinical applications. Bull semen is commonly used in artificial insemination procedures, and microbial contamination of semen can directly impact reproductive success. The presence of resistant bacteria indicates that such contaminations can become more challenging and that traditional treatment methods may be insufficient to control these infections. In this context, there is a need to develop alternative strategies to combat antibiotic resistance and prevent microbial contamination.

While our study contributes to the body of knowledge regarding microbial contamination in bull semen, it

highlights the need for more comprehensive research in this area. Further studies evaluating the effects of different bacterial strains and contamination durations on sperm quality will help improve the understanding of this issue.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: BE, MGSK and CK contributed to the project idea, design and execution of the study. BE and CK contributed to the acquisition of data. BE and VEE analysed the data. BE and CK drafted and wrote the manuscript. All authors reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

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

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RESEARCH ARTICLE

Investigation of Seroprevalence of *Dirofilaria immitis*, *Ehrlichia canis*, *Borrelia burgdorferi* and *Anaplasma spp.* in Dogs Living in Shelter in Şanlıurfa Province

Nursen TOPAL[1*], Mustafa Sinan AKTAŞ[2]

[1] Department of Veterinary Internal Medicine, Ataturk University Health Sciences Institute, Erzurum, Türkiye
[2]Ataturk University Faculty of Veterinary Medicine, Department of Internal Medicine, Erzurum, Türkiye

ABSTRACT

The aim of this study was to determine the seroprevalence of *Ehrlichia canis* (*E.canis*), *Anaplasma spp*, *Borrelia burgdorferi* (*B. burgdorferi)* and *Dirofilaria immitis* (*D. immitis*) in dogs living in shelters in Şanlıurfa province of Turkey. The study material consisted of 100 dogs (76 female and 24 male) living in a shelter in Şanlıurfa province. Sera obtained from blood samples were tested for qualitative detection of *D. immitis* antigen and antibodies to *E. canis*, *B. burgdorferi*, and *Anaplasma* spp with a rapid test kit working with the principle of immunochromatographic analysis. *D. immitis* antigen was not detected in any of the dogs. The presence of antibodies *Anaplasma* spp, *E. canis, and B. burgdorferi* was determined as 65%, 21% and 4%, respectively. The highest *Anaplasma* spp seropositivity was determined in the 2>3≤ years old age group, female and mixed dogs. When seropositivity was compared between age groups, sexes and breeds, the difference was found to be statistically significant ($p<0.05$). The highest *E. canis* seropositivity was observed in the 2>3≤ years old age group, in females and crossbred dogs, and the difference between the sex was statistically significant ($p<0.05$). Similarly, the highest *B. burgdorferi* seropositivity was detected in the 2>3≤ years old age group, in females and crossbred dogs, and the difference between the sex was statistically significant ($p<0.05$). It was concluded that some vector-borne disease such as Anaplasmosis, Ehrlichiosis, and Lyme were seen in dogs living in shelters in Şanlıurfa province and necessary precautions should be taken to combat these diseases.

Keywords: Dog, Seroprevalance, Şanlıurfa province, Vector-borne diseases.
Keywords: Dog, Seroprevalance, Şanlıurfa province, Vector-borne diseases.

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Şanlıurfa'da Barınakta Yaşayan Köpeklerde *Dirofilaria immitis*, *Ehrlichia canis*, *Borrelia burgdorferi* ve *Anaplasma* spp Seroprevalansının Araştırılması

ÖZ

Bu çalışmanın amacı, Türkiye'de Şanlıurfa yöresinde barınakta yaşayan köpeklerde *Dirofilaria immitis* (*D. immitis), Anaplasma* spp, *Ehrlichia canis* (*E. canis*), ve *Borrelia burgdorferi* (*B. burgdorferi*)'nin seroprevalansının belirlenmesidir. Çalışma materyalini Şanlıurfa'da barınakta yaşayan toplam 100 köpek (76 dişi, 24 erkek) oluşturdu. Kan örneklerinden elde edilen serumlar, *D. immitis* antijeni ve *E. canis*, *B. burgdorferi* ve *Anaplasma* spp antikorlarının kalitatif tespiti için immunokromatografik analiz prensibi ile çalışan hızlı test kiti ile test edildi. Köpeklerin hiçbirinde *D. immitis* antijeni belirlenmedi. *Anaplasma* spp, *E. canis ve B. burgdorferi* antikor varlığı sırasıyla %65, %21 ve %4 olarak belirlendi. *Anaplasma* spp seropozitiğinin en yüksek 2>3≤ yaş grubunda, dişilerde ve melezlerde olduğu belirlendi. Seropozitiflik yaş grupları, cinsiyetler ve ırklar arası karşılaştırıldığında, farkın istatistiksel olarak anlamlı olduğu bulundu ($p<0.05$). *E. canis* seropozitifliğinin en yüksek 2>3≤ yaş aralığında, dişilerde ve melez köpeklerde olduğu, cinsiyetler arası farkın istatistiksel olarak önemli olduğu gözlendi ($p<0.05$). Benzer şekilde *B. burgdorferi* seropozitifliğinin en yüksek 2>3≤ yaş aralığında, dişilerde ve melez köpeklerde olduğu, cinsiyetler arası farkın istatistiksel olarak önemli olduğu belirlendi ($p<0.05$). Sonuç olarak anaplasmosis, ehrlichiosis ve lyme gibi bazı vektör kaynaklı hastalıkların Şanlıurfa'da barınakta yaşayan köpeklerde görüldüğü ve bu hastalıklarla mücadele için gerekli tedbirlerin alınması gerektiği sonucuna varıldı.

Anahtar Kelimeler: Köpek, Seroprevalans, Şanlıurfa, Vektör aracılıklı hastalıklar

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ORCID ID; NT: 0009-0001-4204-6985; MSA: 0000-0002-7206-5757
*Corresponding author e-mail: nrsntopal@gmail.com.tr

INTRODUCTION

Anaplasmosis, dirofilariosis, ehrlichiosis and Lyme borreliosis are prevalent vector-borne diseases in dogs globally and have emerged as a significant public health concern in recent years due to their zoonotic nature (Kramer et al., 2014).

The geographical, regional and universal distribution of many diseases with zoonotic importance can be determined by utilising the data obtained from seroprevalence studies. The data obtained is used to determine the spread of diseases and to establish control and eradication programs.In studies conducted to determine the seroprevalence of Anaplasmosis, Ehrlichiosis, Diroflariasis and Lyme borreliosis in dogs in Turkey, the seropositivity rate for *Anaplasma* spp. was 0.5%-52% (Altuğ et al, 2022; Demir and Aktaş, 2020; Karagenç et al., 2005; Smith et al., 2022; Şahinduran and Küçüker, 2018), 0-41.5% for canine monocytic ehrlichiosis (Bingöl, 2019; Bhide et al, 2008; Demir and Aktaş, 2020; Ebani et al., 2014; Güneş et al., 2011; Güneş et al., 2012; Guven et al., 2017; Karagenç et al., 2005; Kırmızıer, 2016; Sarı et al., 2013; Şahindurun and Küçüker, 2018; Ural et al., 2014), 0-38% for *B. burgdorferi* (Altuğ et al., 2022; Düzlü et al., 2014; Kılıç et al. 2013; Littman et al., 2006; Mircean et al., 2017; Uslu, 2008; Vurucu, 2016) and 0-40% for D. immitis (Çakıroğlu and Meral, 2007; Çetinkaya et al., 2016; Demir and Aktaş, 2020; Demirci et al., 2021; Guven et al. 2017; Kozan et al., 2007; Sevgili and Balıkçı, 2005; Sarı et al., 2013; Seven and Akkan, 2023; Şimşek et al., 2008; Yaman et al., 2009; Yıldırım et al., 2007).

A review of the literature revealed two studies on the seroprevalence of D. imitis in dogs in Şanlıurfa. The first was conducted in 2004 (Şahin et al., 2004) and the second in 2013 (Altaş et al., 2013). Additionally, a study was carried out in 2013 to determine the seropositivity of *E. canis* and *B. burgdorferi* (Altaş et al., 2013). No studies were identified that investigated the seroprevalence of *Anaplasma* spp. From these findings, it can be concluded that there is a paucity of research on this topic in the Şanlıurfa region. Accordingly, the objective of the present study was to ascertain the presence of antigens against D. immitis and antibodies against *E. canis*, *Anaplasma* spp. and *B. burgdorferi* in dogs residing in shelters in the Şanlıurfa region of Turkey and to determine the seroprevalence of related diseases.

MATERIALS and METHODS

Animal Material

The study employed a sample of 100 canines temporarily residing in an animal rehabilitation centre in Şanlıurfa (Ethics Committee Decision No. 2022/3). The data set comprised the breed, age and sex of the dogs, as well as their general condition, appetite and interest in their feeding environment and their immediate surroundings.

Blood Sampling

Blood samples were collected once from the dogs through V. cephalica antebrachii into serum tubes (Vacutainer, BD-Plymouth, UK). Given sera samples were separated by centrifugation at 10 min/3000 rpm. Serological analyses were performed immediately on the serum samples obtained

Serological Analysis

For the purpose of conducting serological analyses, a rapid diagnostic test kit (Canivet Tick-4 Combo Rapid Test®, Vet Diagnostix) utilising an immunochromatographic analysis method was employed. The diagnosis was made by determining the antigen against *D. immitis* and the antibodies formed in the presence of *Anaplasma* spp., *E. canis* and *B. burgdorferi* in the analyses performed using serum obtained from blood samples. The rapid diagnostic test kit was utilised in accordance with the manufacturer's instructions.

Statistical Analysis

A Chi-square test was employed to ascertain the statistical significance of the relationship between sex, breed and age in dogs seropositive for *E. canis*, *D. immitis*, *B. burgdorferi* and *Anaplasma* spp. The level of significance was set at $P < 0.05$.

RESULTS

Animal Material Findings

The 100 dogs used in the study and housed at the Şanlıurfa animal rehabilitation centre were found to be in good general condition, with normal interest in nutrition and the environment. Of these dogs, 76 were female, 24 were male, 95 were cross-bred and 5 were greyhounds. Additionally, the presence of ticks was observed in almost all of the dogs.

Dirofilaria immitis

The analyses performed using sera obtained from blood samples taken from the 100 dogs revealed the absence of *D. immitis* antigen.

Anaplasma spp.

The analysis of serum samples from 100 dogs revealed the presence of antibodies against Anaplasma spp in 65 cases, representing a prevalence of 65%. Table-1 presents the data regarding the breed, age, and sex of the dogs that tested positive for Anaplasma spp. antibodies. The seropositivity rate was 40% (10/25) in the 1≤ age group, 72% (18/25) in the 1>2≤ age group, 76% (19/25) in the 2>3≤ age group, and 72% (18/25) in the >3 age group. The seropositivity rate was 47.8% (11/24) among male dogs and 71.1% (54/76) among female dogs. Furthermore, seropositivity was

identified in 64 (68.1%) of the 95 crossbred dogs and only 1 (20.0%) of the 5 greyhounds. Statistically significant differences in seropositivity were observed between age groups ($p < 0.05$), sexes ($p < 0.05$), and breeds ($p < 0.05$).

Ehrlichia canis

The analyses conducted on the serum samples from the canines revealed the presence of antibodies specific to *E. canis* in 21 (21%) of the 100 dogs. Table-2 presents the data regarding the breed, age, and sex of the dogs that tested positive for *E. canis* antibodies. The seropositive dogs were analysed according to age group. The results showed that 1 (4%) of 25 dogs in the 1≤ age group, 5 (20%) of 25 dogs in the 1>2≤ age group, 8 (32.0%) of 25 dogs in the 2>3≤ age group and 7 (28.0%) of 25 dogs in the >3 age group were seropositive. The observed differences in age ranges were found to be statistically insignificant ($p > 0.05$). The seropositivity rate was statistically significantly higher in female dogs (26.3%) compared to males (4.2%) ($p < 0.05$). The prevalence of seropositivity was 20% in the crossbred dogs and 20% in the greyhounds, with no statistically significant difference between the two groups ($p > 0.05$).

Borrelia burgdorferi

The presence of antibodies specific to *B. burgdorferi* was identified in four out of 100 serum samples from dogs. Table 3 presents the data regarding the breed, age, and sex of the dogs that tested positive for *B. burgdorferi* antibodies. While no dogs aged 1 year or younger exhibited positivity, 1% (1/100) of dogs aged 1–2 years and 8% (2/25) of dogs aged 2–3 years were positive. The evaluation of the dogs with seropositivity according to age revealed no statistically significant difference ($p > 0.05$). In the evaluation according to sex, none of the 24 male dogs were seropositive, whereas seropositivity was observed in 4 of the 76 female dogs (5.3%). A statistically significant difference was observed in seropositivity between sexes ($p < 0.05$). In the evaluation according to breeds, seropositivity was identified in four (4.2%) of the 95 crossbred dogs, whereas none (0%) of the five greyhounds exhibited seropositivity. No statistically significant difference in seropositivity was observed between breeds ($p > 0.05$).

Table 1. Breed, Age, and Sex Data of Dogs with Identified *Anaplasma spp.* Antibodies

Breed	Age								Total Positive Number
	1≤		1>2≤		2>3≤		>3		
	Female	Male	Female	Male	Female	Male	Female	Male	
Crossbred (n=95)	8	2	16	2	17	2	13	5	64
Greyhounds (n=5)	-	-	1	-	-	-	-	-	1

Table 2. Breed, Age, and Sex Data of Dogs with Identified *E.canis* Antibodies

Breed	Age								Total Positive Number
	1≤		1>2≤		2>3≤		>3		
	Female	Male	Female	Male	Female	Male	Female	Male	
Crossbred (n=95)	1	-	4	-	8	-	6	1	20
Greyhounds (n=5)	-	-	1	-	-	-	-	-	1

Table 3. Breed, Age, and Sex Data of Dogs with Identified *B.burgdorferi* Antibodies

Breed	Age								Total Positive Number
	1≤		1>2≤		2>3≤		>3		
	Female	Male	Female	Male	Female	Male	Female	Male	
Crossbred (n=95)	-	-	1	-	2	-	1	-	4
Greyhounds (n=5)	-	-	-	-	-	-	-	-	-

DISCUSSION

Among the vector-borne agents in dogs, E. canis, B. burgdorferi, and Anaplasma spp. are the most important bacterial species, and D. immitis is the most important nematode species (Day, 2011).

A substantial body of research has been conducted in Turkey with the objective of determining the prevalence and factors associated with the spread of D. immitis in dogs. In a study conducted in shelters in the Trakya Region, 14.7% in Edirne, 11% in Kırklareli, 1% in Tekirdağ, and 0% in Istanbul (Çetinkaya et al., 2016), 3.6% in Afyonkarahisar, 1.4% in Eskişehir (Kozan et al., 2007), 9.6% in Kayseri (Yildirim et al, 2007), 26% in Hatay (Yaman et al., 2009), 4.4% in Erzurum (Demir and Aktaş, 2020), 40% in Iğdır (Kılıç et al., 2013), 18.3% in Kocaeli, 14.8% in Ankara, 12.3% in Sakarya, 12.3% in Mersin, 10.5% in Mersin, and 0% in Elâzığ (Şimsek et al., 2008). In studies conducted in Turkey, regional evaluations on the subject were also made, and it was reported that the seropositivity rate in dogs ranged from 5.8% to 30% in the Central Anatolia Region, from 9.8% to 17.8% in the Eastern Anatolia Region, from 3.7% to 26% in the Southeastern Anatolia Region, from 13.9% in the Ege Region, and from 0.2% to 1.5% in the Marmara Region (Demirci et al., 2021). A study conducted in north-eastern Turkey revealed a seropositivity rate of 1.5% for D. immitis (Guven et al., 2017). In a study conducted in Şanlıurfa in 2004 to determine the prevalence of D. immitis in dogs, the seropositivity rate was found to be 5.5% in the city centre and 10.5% in the villages connected to the centre (Şahin et al., 2004). In this study, which was also conducted on shelter dogs in Şanlıurfa, no cases of D. immitis seropositivity were identified in the 100 dogs sampled. As with the findings of this study, Çakıroğlu and Meral (2007) also evaluated 100 dogs in their investigation into the prevalence of D. immitis infection in dogs in Samsun and similarly found no evidence of infection. Çakıroğlu and Meral (2007) stated that this does not indicate the absence of D. immitis infestation in Samsun, and that negative results may be obtained in non-endemic areas. The absence of positivity in our study may be attributed to the possibility that the sampled dogs may not have been exposed to the agent, or, as postulated by Çakıroğlu and Meral (2007), the parasite may be present in minute quantities within the host. It can thus be concluded that the results do not indicate the absence or low prevalence of D. immitis infestation in Şanlıurfa. A seropositivity rate of 5.5% in the city centre and 10.5% in the villages connected to the centre (Şahin et al., 2004) lends support to this opinion. Furthermore, it has been asserted that microfilariae may not be discernible in 20–30% of canine infections (Rawlings and Calvert, 1995). It is evident from the aforementioned data that further research is required to ascertain the seroprevalence of D. immitis in dogs within the Şanlıurfa region.

Different results have been obtained in studies on the seroprevalence of Anaplasma spp. in dogs in Türkiye. In a study conducted on 371 dogs in the Ege region, 39% (Karagenç et al., 2005) and 0.8% (Demir and Aktaş, 2020) seropositivity was observed in Erzurum. A study conducted in shelters in Trakya determined the seroprevalence of Anaplasma spp. to be 21.6% (Altuğ et al., 2022). When the data obtained in the present study were evaluated in terms of Anaplasma spp, 65% seropositivity was determined, and this rate was found to be quite high when compared with the data above. In studies conducted to determine the seroprevalence of Anaplasma spp, it has been reported that the number of samples taken, the analytical method employed, and the density of ticks may affect the results obtained (Fonseca et al., 2017). The presence of ticks in almost all of the sampled animals and the favourable environmental conditions for ticks support this view and the high seropositivity obtained. In the evaluation of Anaplasma spp. seroprevalence in dogs, no statistically significant differences have been observed with regard to age, sex, breed or lifestyle (Elhelw et al., 2021; Herrin et al., 2017). Furthermore, additional studies have indicated that seropositivity is more prevalent in older dogs (Egenvall et al., 2000;

Kapiainen, 2016). In their study conducted in Erzurum, Demir and Aktaş (2020) determined that both of the two dogs seropositive to Anaplasma spp. in dogs were older than three years of age. The sex of one was male and the other was female. In this study, an evaluation of seropositivity according to age revealed that the highest positivity was observed in the 2–3≤ age group, with a statistically significant difference. It is plausible that exposure of the dogs in this group to infected ticks may have resulted in the current situation. It has been reported that there is no significant difference in seropositivity between genders (Egenvall et al., 2000; Güneş et al., 2011; Kapiainen, 2016). In contrast with the aforementioned report, the present study has identified a statistically significant higher rate of positivity in females. It is hypothesised that this discrepancy can be attributed to the higher number of female dogs included in the present study. Similarly, although previous studies have indicated that seropositivity is not significantly different between breeds (Elhelw et al., 2021; Herrin et al., 2017), the higher positivity observed in mongrels in the present study, which was statistically significant, is likely attributable to the fact that a considerable proportion of the dogs used in the study were crossbred. Additionally, it is postulated that the favourable climate of the region, the density of the plant population and the presence of tick infestation created conducive conditions for the elevated seropositivity determined against Anaplasma spp. in this study. In studies conducted to determine the seroprevalence of E. canis in Turkey, the prevalence was found to be 14.4%-17.77% in Antalya (Kırmızıer, 2016; Şahınduran and Küçüker, 2018), 4.8% in Diyarbakır (Ural et al., 2014) and 7% in Uşak (Bhide et al., 2008). Furthermore, seropositivity was identified at 41.5% in the Aegean region (Karagenç et al., 2005) and 7% in Aydın (Bıngöl, 2019). In a study conducted on 93 canines in Sinop, the seropositivity rate for E. canis was observed to be 18.28% (Güneş et al., 2012). In a study conducted in Iğdır, the prevalence of the infection was found to be 1% (Kılıç et al., 2013). In a study conducted in Erzurum in north-eastern Turkey, the prevalence of E. canis seropositivity was determined to be 9.8% (Guven et al., 2017). Conversely, Demir and Aktaş (2020) did not detect any seropositivity in the dogs they sampled in their study in Erzurum. In this study, conducted on shelter dogs in Şanlıurfa, a seropositivity rate of 21% against E. canis was determined, which is comparatively high in relation to other studies conducted in Turkey. The potential explanations for the observed variation in seroprevalence rates may be attributed to differences in exposure to tick infestation, the selected population, climate and the preferred diagnostic methods, as previously outlined by Ansari-Mood et al. (2015). In studies conducted to determine the relationship between factors such as breed, age and sex with E. canis seropositivity in dogs, it was reported that seropositivity was higher in older dogs (Piantedosi et al., 2017). Additionally, studies have indicated that seropositivity was higher in dogs younger than six months old (Singh et al., 2014). Conversely, although several studies have indicated that there is no difference between breeds and sexes in seropositivity (Ebani et al., 2014; Guven et al., 2017; Güneş et al., 2012; Tsachev et al., 2006), other studies have suggested that seroprevalence is higher in male dogs (Costa et al., 2007). The data obtained in the present study corroborate the findings of the aforementioned reports, indicating that there is no significant difference between breeds and age groups in terms of seropositivity. An evaluation of seropositivity between sexes revealed a higher prevalence in females, which was found to be statistically significant. It is plausible that the higher prevalence in females is attributable to the greater number of female dogs sampled and/or the higher rate of exposure of females to the agent.

There is a limited body of research on B. burgdorferi in dogs in Turkey, and the available data present a range of findings. Clinical signs are observed in only 5-10% of infected dogs (Sarker et al., 2021). In a study conducted in dogs in Sinop, B. burgdorferi seropositivity was found to be 23.2% (Güneş et al., 2011), 0.7% in the Ege region (Düzlü et al., 2014), the prevalence was reported to be 23.2% in Bursa (Ural et al., 2014), 5.4% in Izmir (Vurucu, 2016), 7% in Samsun (Çakır and Pekmezcı, 2020) and 35% in Aydın (Uslu, 2008). In a study conducted in animal shelters in Trakya, 38% of the dogs tested positive for B. burgdorferi (Altuğ ve ark., 2022). In the study conducted by Altas et al. (2013) in Şanlıurfa, no seropositivity was identified in any of the sampled dogs. The present study revealed a seropositivity rate of 4%, indicating the presence of seropositive dogs in Şanlıurfa, which contrasts with the findings of the previous study. A comparison with the results of studies conducted throughout Turkey revealed that the positivity rate was relatively low. It has been suggested that the low or absent seropositivity observed in this study may be attributed to the number of samples analysed and the prevalence of infected ticks in the area (Altas et al., 2013; Farkas et al., 2014). The low seropositivity observed in the present study may be attributed to the number of samples and the number of infected ticks to which the dogs were exposed. Furthermore, Sarı et al. (2013) reported that their study conducted in the Iğdır region did not detect any positivity against B. burgdorferi in any of the sampled dogs. They interpreted this as evidence of the absence of vector tick species that could potentially transmit B. burgdorferi to dogs in the Iğdır province. Although some studies have indicated a breed predisposition in dogs to B. burgdorferi (Gerber et al., 2007), others have not (Olson et al., 2000). The data obtained in the present study indicated that there was no significant difference in the evaluation of B. burgdorferi seropositive dogs in terms of breed. Conversely, Olson et al. (2000) posited that lifestyle, age and sex do not act as predisposing factors for B. burgdorferi in

canines. In a study conducted by Galluzzo et al. (2020), it was found that the risk was approximately five times higher in males than in females. In the present study, an evaluation of the seropositivity rate according to sex revealed a higher positivity rate in females, which was found to be statistically significant. The elevated positivity rate observed in females is likely attributable to the high number of female animals sampled. Merino et al. (2000) established that all canines exhibiting seropositivity for B. burgdorferi were over one year of age. Farkas et al. (2014) posited that seropositivity increased with age in dogs, attributing this phenomenon to an elevated risk of exposure to the agent with advancing age. In the present study, although no statistical difference was observed when the data were evaluated according to age, it was noted that all positive cases were over one year of age, in accordance with the findings of Merino et al. (2000).

CONCLUSION

In conclusion, based on the serological data obtained from the present study, immunochromatographic rapid test kit analyses conducted on shelter dogs in Şanlıurfa detected antibody presence in 65% of dogs for *Anaplasma* spp., 21% for *E. canis*, and 4% for *B. burgdorferi*. These findings highlight the necessity of implementing protective and control measures to prevent the spread of these vector-borne diseases in the Şanlıurfa region.

Conflict of interest: None of the authors have any conflict of interest to declare.

Authors' Contributions: NT and MSA contributed to the project idea, design and execution of the study. NT contributed to the acquisition of data. MSA reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

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CASE REPORT

Psychogenic Lithophagia in a German Shepherd Dog

Almina GÜNEŞ[1*], Aynur ŞİMŞEK[1], Akın KOÇHAN[1], Ömer Faruk KATANALP[1], Besra ÇAKMAK[1]

[1]Department of Internal Medicine, Faculty of Veterinary Medicine, Dicle University, Diyarbakir, Türkiye

ABSTRACT

Licking, chewing or ingesting non-nutritive substances is defined as pica. Although pica is generally reported to be due to trace element deficiencies, both medical and behavioral causes for pica must be considered. In the current case, it was aimed to report the clinical reflections of lithophagia associated with behavioral disorder in a dog and the haematobiochemical and some radiographic findings. The research material was consisted of a German shepherd dog with symptoms of vomiting and diarrhea. The result of blood hematobiochemistry was not indicative of alterations that could be associated with the pica, while diagnostic imaging revealed the presence of pebbles in the rectum. Blood samples were also examined to determine possible trace element deficiencies, but no significant changes were observed in these parameters. Given that pica, the ingestion of non-edible materials, is a recognized sign of behavioral problems, this aspect must be appropriately investigated, diagnosed and treated.

Keywords: Behavioral disorder, Dog, Lithophagia, Pica

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Bir Alman Çoban Köpeğinde Psikojenik Litofaji

ÖZ

Besin niteliğinde olmayan maddelerin yalanması, çiğnenmesi veya yutulması pika olarak tanımlanmaktadır. Pikanın genellikle iz element eksikliklerine bağlı olarak şekillendiği bildirilse de hem medikal hem de davranışsal nedenler göz önünde bulundurulmalıdır. Mevcut olguda bir köpekte davranış bozukluğuna eşlik eden litofajinin klinik yansımaları ile hematobiyokimyasal ve bazı radyografik bulguların bildirilmesi amaçlanmıştır. Araştırma materyalini kusma ve diyare semptomları olan bir Alman çoban köpeği oluşturdu. Kan hematobiyokimyasının sonucu pika ile ilişkili olabilecek değişiklikleri göstermezken, tanısal görüntüleme rektumda çakıl taşlarının varlığını ortaya çıkardı. Olası iz element eksikliklerini belirlemek için kan örnekleri de incelenmiş, ancak bu parametrelerde önemli bir değişiklik gözlenmemiştir. Yenilebilir olmayan maddelerin yenmesi olarak tanımlanan pika davranışsal sorunların bilinen bir işareti olduğundan uygun şekilde araştırılmalı, teşhis edilmeli ve tedavi edilmelidir.

Anahtar kelimeler: Davranış bozukluğu, Köpek, Litofaji, Pika

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ORCID ID; *AG: 0000-0002-5542-2345 AŞ: 0000-0002-2006-6344 AK: 0000-0003-0199-453X ÖFK: 0000-0003-3353-1871 BÇ: 0009-0003-0000-508X*

*Corresponding author e-mail: almina.gunes@dicle.edu.tr

INTRODUCTION

Ingestion of non-nutritive substances for various reasons in humans and animals is defined as pica, also known as allotriophagy (Kumar et al., 2020; Masson et al., 2021). Individuals with pica are reported to taste or tend to swallow non-food substances, including but not limited to hair (tricophagia), stones (lithophagia), pieces of fabric, and even feces (caprophagia) (Kumar et al., 2020). Animals with pica tend to lick and gnaw almost everything they come into contact with, and are reported to be especially prone to eating soil, bones (osteophagy) and garbage. Female dogs, cats, goats, cattles and buffaloes can also act placenta eating (placentophagy) behavior. Although this condition in dogs may be related to protein deficiency, it should not be forgotten that it is a behavioral adaptation developed by the mother to protect the newborn from predators in the environment (Firyal, 2007).

Although the etiology of pica has not been fully elucidated, it is stated that it may develop due to deficiencies of some amino acids, vitamins and trace elements, as well as hunger or changes in mood (boredom) are also effective in the emergence of this disorder (Eyarefe, 2016; Kochan et al., 2023). It has also been reported that hypersensitivity-hyperactivity syndrome in dogs may be a behavioral problem associated with pica and thus foreign body ingestion (Merola and Giussani, 2010). Some researchers suggest that a number of factors such as anxiety, oral obsessive-compulsive disorder , or the owner's work routine (e.g., attachment problems) may cause foreign body ingestion (Masson et al., 2021). While pica in dogs is a common complaint in behavioral consultations, it can also result in situations that can negatively affect the dog's welfare (e.g, punishment or isolation) (Masson et al., 2021).

Due to all the abovementioned, pica in dogs is a subject that should be emphasized, and its causes should be investigated. This study aims to investigate the hematobiochemical, radiographic, and behavioral aspects of psychogenic lithophagia in a German shepherd dog.

CASE PRESENTATION

The research material consisted of a 1.5-year-old intact female German shepherd mine detection dog with a body weight of 18.5 kg with complaints of vomiting and diarrhea. It was learned that the symptoms of vomiting and diarrhea were observed by the dog's owner for a long time without any abnormal findings such as loss of appetite, depression, poor performance or a change in diet. The dog with lithophagia previously had another owner, and the previous owner stated that the patient had been showing the same symptoms approximately for 9 months and that the sibling of the dog with lithophagia also suffered from pica. It has also been reported recently that pebbles have been seen in the stomach and intestinal contents

and that the dog tends to swallow all kinds of non-nutritive substances, while on duty, especially pebbles. A detailed examination was started by obtaining informed consent from the owner.

On physical examination, it was determined that mucosa and conjunctiva appeared mild hyperemic, respiratory rate was 28 breaths/minute, pulse rate was 96 beats/minute, and rectal temperature was 38.6°C. Increased sensitivity was observed during abdominal palpation of the mesogastric area. During the physical examination, it was noted that the dog could not be restrained without the assistance of the owner. Considering the anamnesis, digital disimpaction was performed and numerous pebbles were detected in the rectum. Laterolateral radiographic images of the abdomen were taken to determine the amount and location of the pebbles. Subsequently, an ultrasonographic examination was performed to evaluate the intestinal wall.

To perform serum biochemical and hematological analysis, blood samples were taken from the vena cephalica antebrachii into tubes with and without anticoagulant. The blood sample taken into an anticoagulant tube was analyzed immediately with a blood counting device (Mindray BC-2800Vet, China). The blood sample collected in a tube without anticoagulant was centrifuged at 3000 revolutions per minute for 10 minutes after clotting at room temperature, and the serum sample obtained was analyzed using biochemistry devices (Fujifilm, Dri-Chem NX500, Japan; Abbott, Architect C16000, USA).

A decrease in hematocrit value and microcytosis were detected in the complete blood count compared to reference ranges (Table 1). Compared with the reference ranges of the serum biochemical analysis results showed that the serum globulin level decreased (Table 2). Serum iron, unsaturated iron binding capacity, total iron binding capacity, zinc and copper levels are shown in Table 3. On the L/L image of the abdomen, mild ventral displacement of the column detected due to numerous pebble ingestion (Figure 1). Ultrasonographic examination revealed increased echogenicity in the serosa layer of jejenum (Figure 2).

Since the determination of serum iron, unsaturated iron binding capacity, total iron binding capacity, zinc and copper levels takes time and it is known that pica is usually associated with trace element deficiencies (Eyarefe, 2016; Firyal, 2007; Kochan et al., 2023; Kumar et al., 2020), supportive treatment initiated without waiting for the complete results of laboratory analysis. It has been reported that excessive faecal vitamin D3 loss may occur in gastrointestinal system diseases of dogs (Dittmer and Thompson, 2011), and that the addition of vitamin B12 and vitamin D3 to

the treatment protocol may be beneficial (Tamura, 2025). Similarly, it has been reported that faecal zinc loss may occur in dogs with gastroenteritis (Pereira et al., 2021).

Table 1: Hematological findings of a dog with lithophagia

Parameters	Results	Reference ranges (Turgut, 2000)
WBC (10^9/l)	14.8	6.0 – 17.0
Lenf (10^9/l)	3.8	0.8 – 5.1
Mon (10^9/l)	0.9	0.0 – 1.8
Gra (10^9/l)	10.1	4.0 – 12.6
Lenf (%)	25.5	12.0 – 30.0
Mon (%)	6.3	2.0 – 9.0
Gra (%)	68.2	60.0 – 83.0
RBC (10^{12}/l)	6.4	5.5 – 8.5
HGB (g/dl)	13.8	11.0 – 19.0
HCT (%)	38.2	39.0 – 56.0
MCV (fl)	59.7	62.0 – 72.0
MCH (g/dl)	21.5	20.0 – 25.0
MCHC (fl)	36.1	30.0 – 38.0
RDW (%)	12.8	11.0 – 15.5
PLT (10^9/l)	388	117 – 460
MPV (fl)	7.2	7.0 – 12.9
Eos (%)	1.7	1.0 – 10.0

WBC: total leukocyte count, Lenf: lymphocyte, Mon: monocyte, Gran: granulocyte, RBC: total erythrocyte count, HGB: hemoglobin, HCT: hematocrit, MCV: mean erythrocyte volume, MCH: mean erythrocyte hemoglobin, MCHC: mean erythrocyte hemoglobin concentration, RDW: red cell distribution, PLT: platelet, MPV: mean platelet volume, Eos: eosinophil

Table 2: Serum biochemical analysis findings of a dog with lithophagia

Parameters	Results	Reference ranges (Turgut, 2000)
ALT (IU/l)	44	10-88
AST (IU/l)	35	10-88
ALP (IU/l)	48	20-150
GGT (IU/l)	10	1.0-10
BUN (mg/dl)	17	12-25
Cre (mg/dl)	0.96	0.5-1.5
TP (g/dl)	4.7	5.4-7.4
ALB(g/dl)	2.6	2.3-3.8
Glob (g/dl)	2.1	2.3-5.2
Amyl (IU/l)	760	300-2 000
LIP (IU/l)	67	25-750
GLU (mg/dl)	78	60-110
IP (mg/dl)	5.2	2.2-5.5
Mg (mg/dl)	1.7	1.2-2
Ca (mg/dl)	10.4	8.6-11.2
Na (mEq/l)	146	141-153
K (mEq/l)	4.9	3.7-5.8
Cl (mEq/l)	111	105-115

ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, GGT: gamma glutamyl transferase, BUN: blood urea nitrogen, Cre: creatinine, Amyl: amylase, LIP: lipase, GLU: glucose, TP: total protein, ALB: albumin, Glob: globulin, IP: phosphorus, Mg: magnesium, Ca: calcium, Na: sodium, K: potassium, Cl: chlorine

In light of these findings, a treatment plan was devised, involving the administration of zinc sulfate heptahydrate (Zinco®; Berko İlaç) twice daily per os (PO), at a dose of 3 ml, butaphosphan and vitamin B12 (Symphos®; IPM İlaç) every other day by subcutaneous (SC) injection at a dose of 1.5 ml, and vitamin D3 (Devit-3®; Deva) every other day PO, at a dose of a single drop, for a duration of 14 days.

After the treatment was terminated, the owner was called, and an anamnesis was taken regarding the dog's current condition. It was reported that the dog continued to show pica symptoms and still has diarrhea following lithophagia. It also learned that the dog was kept away from potential foreign bodies that could be ingested by changing its shelter, but despite this, the dog licked and gnawed the shelter material and showed lithophagia behavior again while on duty. The fact that the laboratory results were within reference ranges and the persistence of lithophagia suggested that this condition might be due to a behavioral disorder. Therefore, the 4A grid scoring (Masson et al., 2021) for dogs was used to assess behavioral disorder based on information obtained from the owner. In this context, aggressivity, anxiety, attachment and autocontrol were evaluated and each of them was scored as 12, 12, 15 and 17 out of 20, respectively. Therefore, it was concluded that the lithophagia in the present case occurred due to a behavioral disorder.

Table 3: Serum trace element levels in the dog with lithophagia

Parameters	Results	Reference ranges
Fe (µg/dl)	137	94.7-223.4 (Silvestrini et al., 2014)
UIBC (µg/dl)	186	184.3-307.2 (Silvestrini et al., 2014)
TIBC (µg/dl)	319	318.4-480.4 (Silvestrini et al., 2014)
Zn (µmol/l)	25.3	4.9-19.7 (Zentrichová et al., 2023)
Cu (µg/dl)	46.6	20-80 (Amundson et al., 2024)

Abbreviations: Fe: iron, UIBC: unsaturated iron-binding capacity, TIBC: total iron-binding capacity, Zn: zinc, Cu: copper

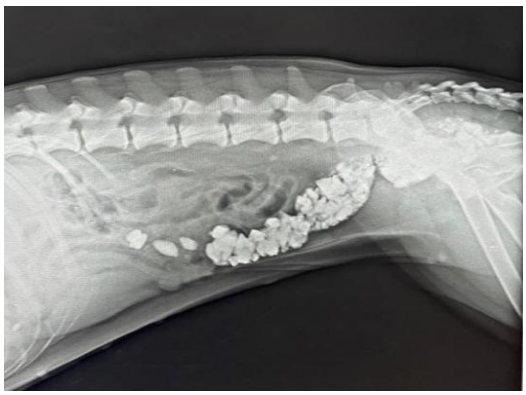

Figure 1: Laterolateral image of the abdomen, mild ventral displacement of the column due to numerous pebbles ingestion.

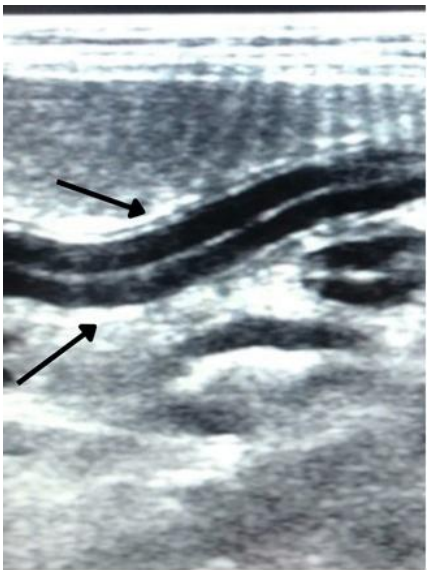

Figure 2: Sagittal plane image of the right dorsal abdomen of the dog with lithophagia. Arrows: Increased echogenicity in the serosa layer of jejenum.

DISCUSSION

The causes of pica remain unexplored, but it may occur due to certain amino acid, vitamin and trace element deficiencies or as a behavioral disorder (Eyarefe, 2016; Kochan et al., 2023). In this study, it was determined that pica was caused by behavioral disorder in accordance with the reports of the researchers (Masson et al., 2021).

In human pica cases, the risk of lithophagia may increase in individuals with a family history of pica (Rajendra et al., 2016). Therefore, in addition to behavioral disorders, family history is a factor that should be taken into consideration when investigating underlying causes of pica in dogs. The pica in the present case was found to be related to behavioural disorder as reported by the researchers (Eyarefe, 2016; Masson et al., 2021; Rajendra et al., 2016). Although the current study was limited by the inability to determine all trace elements and vitamin levels and the inability to collect data from the other dog reported in the anamnesis, the fact that the dog was fed a good quality, balanced commercial diet and pica was reported in family members supports that this phenomenon developed due to behavioral disorder.

In a study (n=42), researchers (Masson et al., 2021) found that fabric (54.8%), plastic (28.6%), rubber (21.4%), stone (4.8%), metal (4.8%) and wood (2.4%) were the most common materials ingested by dogs. It has also been reported that some dogs tend to swallow multiple foreign body (Masson et al., 2021). In this case the dog was found to be prone to ingesting stones. However, it is important to be aware that any foreign body can be ingested.

It has been reported that the clinical findings encountered in dogs with pica are pain, cramps, constipation or diarrhea (Firyal, 2007; Kochan et al., 2023) and common complications include gastrointestinal obstruction, intestinal necrosis, perforation, peritonitis and hypovolemia (Masson et al., 2021). In this case, pain on abdominal palpation and diarrhea due to lithophagia were detected, but no complications were encountered. Authors believe that in addition to the quantity, shape and size of the foreign bodies taken, the time elapsed since ingestion and the consciousness level of the patient owners are also effective in the formation of complications.

In the hematological findings of the dog with lithophagia, a decrease in hematocrit value and microcytosis were detected. Since all erythrogram indices must be evaluated together, there is no significant decrease in the parameters, and there are individual differences (Turgut, 2000), changes in hematological findings were not associated with lithophagia and were not found to be significant. Serum biochemistry showed a decrease in serum globulin levels. It has been highlighted that serum globulin levels may decrease in canine lymphocytic plasmacytic enteritis (Craven and Washabau, 2019). Therefore, the authors thought that the decrease in serum globulin levels in the present case was related with enteritis. To the authors' knowledge, it was determined that there were serious differences in the reference ranges specified for serum zinc levels in dogs (Mert et al., 2008; Tomza-Marciniak et al., 2012; Zentrichová et al., 2023). Researchers reported that this difference may be related to the number of animals used in the study, the diet applied, breed and gender (Zentrichová et al., 2023). In the same study, they emphasized that the most important factor was gender and reported that serum zinc levels were significantly higher in intact females than intact males (Zentrichová et al., 2023). In the present case, although it was noted that the serum zinc level was high compared to the reference range, this was considered normal as the dog was an intact female.

CONCLUSION

Pica, defined as licking, chewing or ingesting non-food items, is a significant life-threatening problem with potential gastrointestinal complications and a direct negative impact on the dog's welfare. Behavioral consultation of dogs with a tendency to swallow foreign bodies is an area that needs to be expanded, although it is rare in veterinary practice. It should not be forgotten that pica may be of psychogenic origin, especially in dogs with a family history of pica, as in the present case, and those that have received strict training to be used in different services, and the incidence of pica in such service dogs may be higher compared to other dogs. It is also thought that in cases where pica is observed, the environment should be arranged according to the needs of the dog, potential foreign bodies that may be ingested should be eliminated, and patient owners should be made aware of this issue. It was concluded that behavioral disorders in dogs exhibiting pica despite being fed a balanced diet and having a normal hematobiochemical profile is an issue that needs to be addressed.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: AŞ and AK contributed to the project idea, design and execution of the study. AG, ÖFK and BÇ contributed to the acquisition of data. AŞ, AK and ÖFK analysed the data. AG drafted and wrote the manuscript. AŞ and AK reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

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

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CASE REPORT

Case Report: Lily (*Lilium Orientalis*) Poisoning in a Cat

Zeynep BİDECİ[1]*, Emre ERDOĞAN[2], Sena N. KİMYA[2], Turan CİVELEK[1]

[1]*Department of Internal Medicine, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Türkiye*
[2]*Florya Doğa Veterinary Clinic, İstanbul, Türkiye*

ABSTRACT

Some lily species are toxic to cats. The main harmful effect is on the kidneys and leads to acute renal damage. Ingestion of any part of the plant can cause poisoning. Even the ingestion of small amounts of plant parts can have serious consequences. The mechanism of toxicity is not known exactly. Symptoms develop rapidly. In the progress of the disease, the gastrointestinal system is primarily affected. Afterward, polyuria, dehydration, and renal failure accompany the symptoms. Seizures may occur in severe cases. In acute cases, the animal may be tried to induce vomiting, and/or applications to reduce toxin absorption may be made. Renal perfusion is attempted to be provided with intravenous fluid applications. Treatment options are more limited in the cases of lily intoxication if renal failure develops. Taking precautions against the plant is more effective than treatment. Therefore, it is important to raise awareness of cat owners. In the present case, stargazer poisoning in an elderly female British Shorthair cat brought to a private veterinary clinic for examination and treatment was discussed. Toxication was diagnosed based on clinical, hematological, and biochemical findings. The cat presented with symptoms such as vomiting, anorexia, lethargy, and urinary obstruction, all indicative of lily toxicosis. This case report aims to emphasize the toxicity that may be caused by lily plants in cats living at home. Also, it provides information about diagnostic and therapeutic procedures.

Key Words: Feline, Nephrotoxicity, Plant, Toxication, Toxicity

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Vaka Raporu: Bir Kedide Zambak (*Lilium Orientalis*) Zehirlenmesi

ÖZ

Bazı zambak türleri kediler için toksiktir. Başlıca toksik etkisi böbrekler üzerinde görülür ve akut renal hasara yol açar. Bitkinin herhangi bir kısmının alınması zehirlenmeye neden olabilir. Az miktarda bitki parçası yutulduğunda dahi ciddi sonuçlarla karşılaşılabilir. Toksisitenin mekanizması tam olarak bilinmemektedir. Belirtiler hızla gelişir. Hastalık seyrinde öncelikle gastrointestinal sistem etkilenir, sonrasında tabloya poliüri, dehidrasyon ve böbrek yetmezliği eşlik eder. Şiddetli vakalarda nöbet görülebilir. Akut olgularda hayvan kusturulmaya çalışılabilir ve/veya toksin emilimini azaltıcı uygulamalar yapılabilir. İntravenöz sıvı uygulamaları ile renal perfüzyon sağlanmaya çalışılır. Böbrek yetmezliği gelişmiş toksikasyon vakalarında tedavi seçenekleri daha sınırlıdır. Bitkiye karşı önlem almak tedaviden daha etkilidir. Bu nedenle kedi sahiplerinin bilinçlendirilmesi önemlidir. Sunulan raporda özel bir veteriner kliniğe muayene ve tedavi amaçlı getirilen bir yaşlı dişi british shorthair ırkı kedide stargazer zehirlenmesi konu edildi. Anamnezde kusma, iştahsızlık, durgunluk ve idrar yapamama şikayeti bulunan kediye klinik, hematolojik ve biyokimyasal bulgular ve anamnez bilgi dahilinde zambak toksikasyonu tanısı kondu. Bu olgu sunumu; evde yaşayan kedilerde zambak bitkisinin neden olabileceği toksikasyona dikkat çekmeyi ve yanı sıra tanı ve tedavi prosedürü hakkında bilgi vermeyi hedeflemektedir.

Anahtar Kelimeler: Bitki, Kedi, Nefrotoksisite, Toksikasyon, Toksisite

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ORCID ID; *ZB: 0009-0004-0663-9128, EE: 0009-0005-0324-3950, SNK: 0009-0007-8737-8354, TC: 0000-0002-4819-3484*
*Corresponding author e-mail: zbideci@aku.edu.tr

INTRODUCTION

Cases of plant toxicity in cats and dogs are common because most of them live at home and have occasional access to streets, parks, and rural areas. Ornamental plants found in gardens and widely used for home decoration are responsible for many of the poisonings (Andrade, 2011).

Although there are many lily species, two of them cause significant nephrotoxicity in cats. These are *True Lilies (Lilium ssp.) and Day Lilies (Hemerocallis ssp.)*. Even the ingestion of a small plant part can be toxic to cats. On the other hand, some species are not toxic (Fitzgerald, 2010). The exact mechanism of action of lily-induced nephrotoxicity is unknown. However, the rapid onset of clinical signs after ingestion of the poisonous species indicates a rapid rate of absorption (Hall, 2006). The lily-induced nephrotoxic syndrome has only been observed in cats. No age, sex or breed-related predisposition has been identified. Rats, mice, and rabbits consume up to 1.5 times their body weight in lilies, yet no nephrotoxic damage is observed (Hall, 2007). On the other hand, similarly, lily does not produce nephrotoxic effects even if consumed in large amounts by dogs. Only vomiting and gastrointestinal symptoms are observed (Fitzgerald, 2010).

Clinical signs of lily poisoning in cats include hypersalivation, vomiting, anorexia, lethargy, polyuria, polydipsia, azotemia, glucosuria, proteinuria, and in severe cases oliguria/anuria (Rumbeiha et al., 2004). The expected course of poisoning is as follows: 1-3 hours after ingestion lily causes decreased appetite, hypersalivation, vomiting, and depression in cats. Vomiting and hypersalivation may persist for 2 to 6 hours. Gastrointestinal signs subside after about 6 hours. At 12-30 hours, polydipsia and polyuria are observed due to progression of kidney damage. This is followed by dehydration which further increases the effects of kidney damage. Within 24 to 48 hours, the condition progresses to an oliguric phase, followed by an anuric phase. When renal activity stops, metabolic wastes accumulate in the body and cause the resumption of vomiting. Asthenia usually develops 30-72 hours after lily consumption. In 3-7 days, the symptoms gradually worsen and the case ends in death (Fitzgerald, 2010). Apart from the nephrotoxic effect, pancreatic damage and seizures may also be observed (Rumbeiha et al., 2004).

There is no antidote or specific treatment for this intoxication. If the condition is recognised early, supportive treatment can be given to manage symptoms and provide a chance of recovery. Successful treatment involves initiating fluid diuresis before the onset of anuric renal failure (Bates, 2006). Since drugs such as furosemide and mannitol used to stimulate urine output when anuria develops are not very successful in treatment, peritoneal dialysis or hemodialysis is thought to be the only potential treatment (Tefft, 2004; Hall, 2013). However, in one case, oligo-anuric acute kidney injury after lily toxicity was reported to be resolved by medical management (To et al., 2023).

CASE HISTORY

The material of this study was a 1-year-old female British Shorthair cat. In the anamnesis, it was learned that the cat had eaten a house plant (lily) approximately 21 hours before the first clinic application and had been taken to a veterinary clinic (first clinic) with the complaint of vomiting. It was also learned that; at this initial clinic, the patient was evaluated clinically, hemogram and biochemistry analyses as well as radiographically and was treated in accordance with the following protocol. The results of the hemogram and biochemical analyses associated with this initial presentation are given in Tables 1 and 2. According to the data provided by the history; intravenous balanced solution, H2-receptor antagonist, antihistamine, choleretic and amino acid solution, as well as antiemetic and nonsteroidal anti-inflammatory was applied at the first clinic. After the treatment, the vomiting stopped and the patient was discharged. The cat, which symptoms reappeared about 12 hours later, was brought to another veterinary clinic (*Florya Doğa Veterinary Clinic, İstanbul, Türkiye*) approximately 24 hours after being discharged from the first clinic with complaints of anuria, loss of appetite, difficulty breathing, and recurrent vomiting. Blood analyses (Tables 3 and 4) and radiographic examination were repeated immediately. The patient was evaluated ultrasonographically. Emergency dialysis was considered for the patient whose treatment was started with slow fluid replacement subcutaneously. However, due to the delay in the case, our patient, whose general condition was quite poor and whose respiration was depressed, died after a short time despite intubation.

Ultrasonographic examination revealed bilateral perinephric fluid collection (Figure 1). Resistive index measurements were 0.79 for the right kidney and 0.79 for the left kidney (Figure 2). The empty urinary bladder could not be evaluated. Radiography revealed ascites in the abdomen as well as pulmonary edema (Figure 3). Biochemical analyses demonstrated elevated levels of creatinine, ALT, BUN, glucose, calcium, phosphorus, and total bilirubin, while chloride, sodium, and total protein levels were found to be decreased (Table 3). Hemogram results showed that RBC, HGB, MCHC, and neutrophil values were higher than references (Table 4).

Table 1. Hematological findings (initial clinical results)

Parameter	Result	Reference Range (Fielder, 2024)	Unit
WBC	9.15	5.5-19.5	$x10^9/L$
Neutrophils	7.99	1.8-12.6	$x10^9/L$
Neutrophils	87.3	45.0-64.0	%
Eosinophils	0.38	0.0-0.8	$x10^9/L$
Eosinophils	4.2	0.0-4.0	%
Lymphocytes	0.47	1.5-7.0	$x10^9/L$
Lymphocytes	5.1	27.0-36.0	%
Monocytes	0.31	0.0-0.9	$x10^9/L$
Monocytes	3.4	0.0-5.0	%
RBC	11.28	5.0-10.0	$x10^{12}/L$
Hemoglobin	17.0	9.8-15.4	g/dL
MCV	40.5	39.0-55.0	fL
MCH	15.1	13.0-17.0	pg
MCHC	37.3	30.0-36.0	g/dL
Hematocrit	45.7	26.0-51.0	%
Platelets	427	100.0-518.0	$x10^9/L$
MPV	11.0	12-18	fL

*WBC, White Blood Cell count; RBC, Red Blood Cell count; MCV, Mean Corpuscular Volume; MCH – Mean Corpuscular Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; MPV, Mean Platelet Volume

Table 2. Biochemistry measurement data (initial clinical results)

Parameter	Result	Reference Range (Fielder, 2024)	Unit
ALP	28	0-45	U/L
ALT	482	25-97	U/L
BUN	111.6	19-34	mg/dL
BUN/CRE	11.4	4.0-33.0	mg/dL
CRE	9.83	0.9-2.2	mg/dL
GLU	132	60.0-120.0	mg/dL
TP	6.7	6.0-7.9	g/dL

*ALP, Alkaline Phosphatase; ALT, Alanine Aminotransferase; BUN, Blood Urea Nitrogen; BUN/CRE, Blood Urea Nitrogen / Creatinine Ratio; CRE, Creatinine; GLU, Glucose; TP, Total Protein

Table 3. Biochemistry measurement data (the data from the second clinic)

Parameter	Result	Reference Range (Fielder, 2024)	Unit
Cl^-	94.00	115.00 - 130.00	mEq/L
K^+	5.30	3.40 - 4.60	mEq/L
Na^+	127.00	146.00 - 156.00	mEq/L
Na/K	23.96	0.00-0.00	mEq/L
CRE	15.33	0.9-2.2	mg/dL
ALB	2.50	2.80 - 3.90	g/dL
ALB/GLB	0.83	0.35 - 1.50	g/dL
ALP	40.00	0.00 - 45.00	U/L
ALT	208.00	25.00 - 97.00	U/L
GGT	10.00	1.00 - 10.00	U/L
GLU	169.00	60.00 - 120.00	mg/dL
BUN	140.00	19.0-34.0	mg/dL
BUN/CRE	9.13	4.00 - 33.00	mg/dL
Ca^{++}	12.00	8.70 - 11.70	mEq/L
IP	15.00	3.0 - 6.10	mEq/L
TBIL	1.10	0.0 - 0.10	mg/dL
TCHO	133.00	71.0-156.0	mg/dL
TP	5.50	6.0 - 7.90	g/dL

*Cl^-, Chloride; K^+, Potassium; Na^+, Sodium; Na/K, Sodium/Potassium Ratio; CRE, Creatinine; ALB, Albumin; ALB/GLB, Albumin/Globulin Ratio; ALP, Alkaline Phosphatase; ALT, Alanine Aminotransferase; GGT, Gamma-Glutamyl Transferase; GLU, Glucose; BUN, Blood Urea Nitrogen; BUN/CRE, Blood Urea Nitrogen/Creatinine Ratio; Ca^{2+}, Ionized Calcium; IP, Inorganic Phosphorus; TBIL, Total Bilirubin; TCHO, Total Cholesterol; TP, Total Protein

Table 4. Hematological findings (the data from the second clinic)

Parameter	Result	Reference Range (Fielder, 2024)	Unit
WBC	14.33	5.5-19.5	$x10^9/L$
Neutrophil	11.65	1.8-12.6	$x10^9/L$
Neutrophil	81.30	45.0-64.0	%
Eosinophil	0.46	0.0-0.8	$x10^9/L$
Eosinophil	3.20	0.0-4.0	%
Lymphocyte	1.63	1.5-7.0	$x10^9/L$
Lymphocyte	11.40	27.0-36.0	%
Monocyte	0.44	0.0-0.9	$x10^9/L$
Monocyte	3.10	0.0-5.0	%
RBC	11.93	5.0-10.0	$x10^{12}/L$
Hemoglobin	16.90	9.8-15.4	g/dL
MCV	36.80	39.0-55.0	fL
MCH	14.20	13.0-17.0	pg
MCHC	38.50	30.0-36.0	g/dL
Hematocrit	43.90	26.0-51.0	%
Platelets	377.00	100.0-518.0	$x10^9/L$
MPV	10.80	12-18	fL

*WBC, White Blood Cell count; RBC, Red Blood Cell count; MCV, Mean Corpuscular Volume; MCH – Mean Corpuscular Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; MPV, Mean Platelet Volume

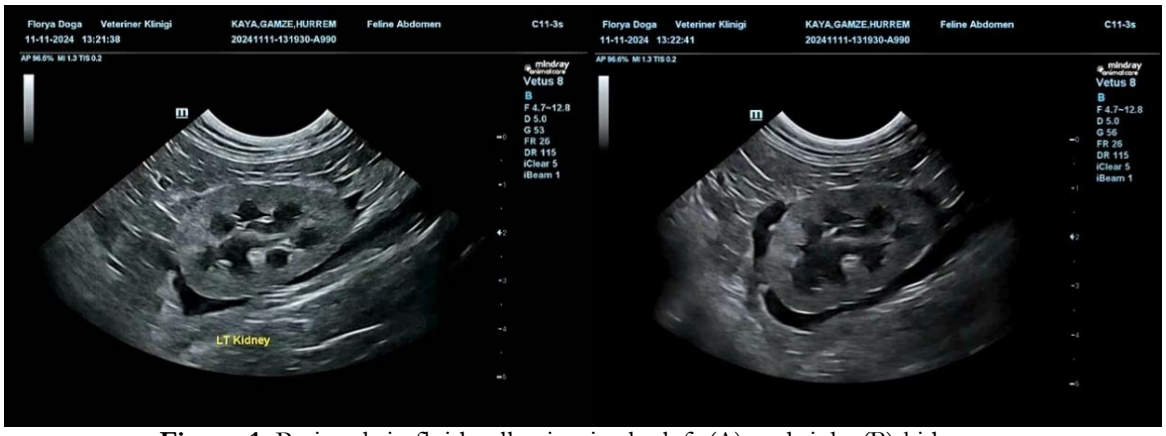

Figure 1: Perinephric fluid collection in the left (A) and right (B) kidneys.

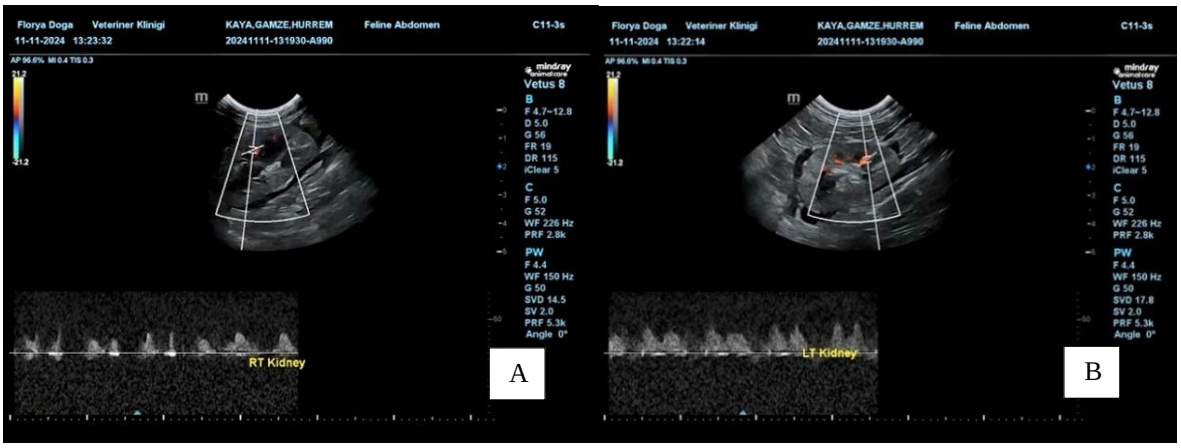

Figure 2: Resistive index measurement in the right (A) and left (B) kidneys.

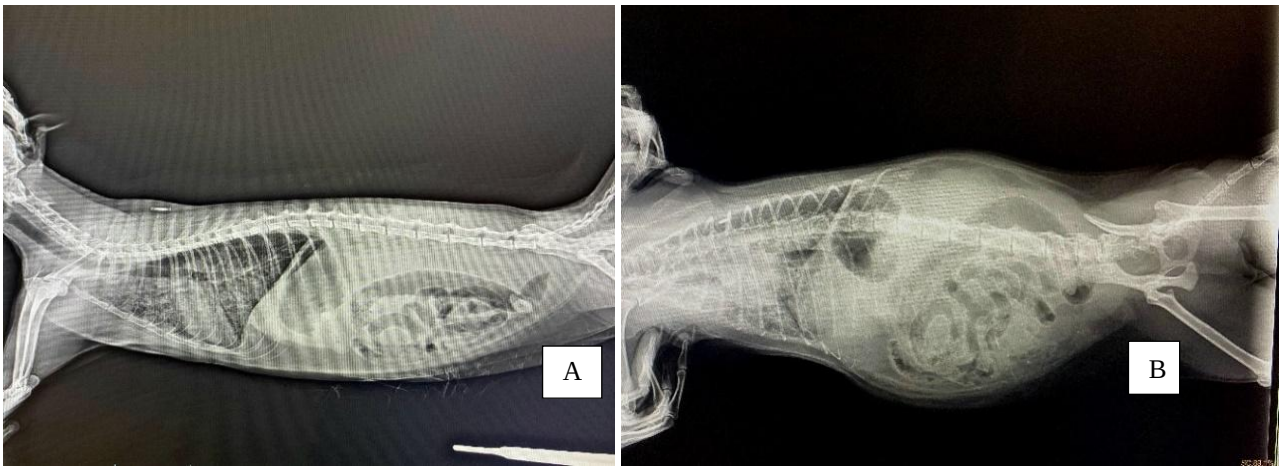

Figure 3: Appearance consistent with pulmonary edema (A) and ascites (B) on radiographs.

DISCUSSION

Cases of plant-based poisoning in cats are frequently encountered in domestic environments. Especially Lilium spp. and Hemerocallis spp. are widely used decorative plants in homes and gardens. All parts of the lily plant are dangerous for cats. Symptoms of toxicity occur rapidly after ingestion (Fitzgerald, 2010). These symptoms include vomiting, lethargy, loss of appetite, and polydipsia. The clinical findings observed in the present case are similar to those reported in the literature. In this case, considering the patient history and the time spent in the first clinic, it was evaluated that anuria probably developed between 40 and 50 hours after ingestion of the plant. Again; BUN, creatinine as well as potassium levels, which are used to determine the current degree of renal effects, were observed to be increased in our case. Creatinine elevation in cats is primarily related to glomerular filtration rate, commonly due to kidney injury, dehydration, or urethral obstruction. In lily toxicosis, marked creatinine rise secondary to acute tubular necrosis, which results in severe renal dysfunction (Hall, 2007). The serum creatinine level, which is the most important parameter that helps to determine lily intoxication, increased independently of the BUN level in this case (Hall, 2004). In the ultrasonographic examination, the resistive index value for both kidneys was 0.79. In cats, values of 0.70 and above are considered pathological (Debruyn et al., 2012). An increased index indicates an increase in vascular resistance similar to renal arterial stenosis and a marked renal disease (Granata et al., 2009). However; ALT activity has the highest sensitivity (more than 80%) for hepatic disorders in cats (Kozat and Sepehrizadeh, 2017). Cats are generally predisposed to develop hepatobiliary pathologies associated with stress and weight loss (Oikonomidis and Milne, 2023). It was evaluated that marked lethargy and hepatic stress may be related to the increase in ALT serum level (Hall, 2013) in this case.
Pulmonary edema, which has been reported in some cases, was also observed in our case (Langston, 2002; Fitzgerald, 2010).
In the presented case, it was evaluated that the treatment applied to the patient at first clinical application and the cessation of vomiting could possibly be related to the fact that the applied treatment was probably more symptomatic and coincided with the beginning and middle stages of the case. This cat, which complaints recurred after a while and entered the anuric phase, probably already had acute renal failure before applying to another clinic for the second time. However, despite these efforts, the patient died. In contrast to the present case, Langston (2002) reported that three out of six cats survived following lily toxication. However, all of these survivors developed chronic kidney disease and required long-term management, including renal-supportive diets such as protein-restricted formulations. The remaining three cats in that study either died or were euthanized.

CONCLUSION

In conclusion, lily poisoning is an extremely critical and life-threatening condition for cats, which must be clinically acknowledged and urgently treated. For the successful management of similar cases, it is of utmost importance for cat owners to provide accurate and timely history to the veterinarian and for veterinary clinics to be knowledgeable about the condition. There is currently no specific or definitive treatment for this toxicity, which leads to a poor prognosis.
The key to the successful treatment of lily toxicity is the preservation of renal function. For this purpose, rapid decontamination and fluid diuresis should be initiated. Early intervention results in a better prognosis. However, in delayed treatment, the chances of recovery are minimal. Prevention is the most effective strategy. To minimize risk, lilies should be kept out of reach in households with cats, or ideally, lilies should not be present at all. Cat owners should prefer non-toxic plants in their living environments, which would be the most appropriate approach.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: TC and ZB contributed to the project idea, design and execution of the study. SNK and EE contributed to the acquisition of data. TC analysed the data. ZB and SNK drafted and wrote the manuscript. TC, ZB and SNK reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

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

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