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The effect of cefquinome on hematological and biochemical parameters following repeated subcutaneous administrations in sheep

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Abstract: The objective of this study was to evaluate the effect of cefquinome on hematological and biochemical parameters following repeated subcutaneous administrations in sheep. The study was conducted with six clinically healthy Merino sheep with age and body weight of 1.5 ± 0.2 years and 39 ± 2.7 kg, respectively. Cefquinome was administered subcutaneously to the sheep once a day for five days at a dose of 2.5 mg/kg. Blood samples were collected by jugular venipuncture prior to drug administration (0 h) and at 48 h and 120 h following the first drug administration. Blood samples were analyzed to determine hematological and biochemical parameters. Hematological and biochemical parameters did not alter following repeated subcutaneous administration of cefquinome ($P > 0.05$). These results indicate that cefquinome could be safe and well-tolerated following repeated subcutaneous administrations of 2.5 mg/kg once daily for five consecutive days in sheep. However, there is a need for molecular and histopathological investigation of the safety of cefquinome after repeated administration in sheep at different doses and administration routes.
Keywords: Biochemistry, cefquinome, hematology, sheep

Koyunlarda tekrarlanan subkutan uygulamaları takiben sefkuinomun hematolojik ve biyokimyasal parametrelere etkisi

Özet: Bu çalışmanın amacı, koyunlarda tekrarlanan subkutan uygulamaları takiben sefkuinomun hematolojik ve biyokimyasal parametreler üzerindeki etkisini değerlendirmektir. Çalışma klinik olarak sağlıklı, 1,5 ± 0,2 yaş ve 39 ± 2,7 kg canlı ağırlığa sahip, altı adet Merinos koyunu üzerinde gerçekleştirildi. Sefkuinom koyunlara 2,5 mg/kg dozda günde bir defa beş gün boyunca deri altı yolla uygulandı. Kan örnekleri ilaç uygulaması öncesi (0. saat) ve ilk uygulamayı takiben 48. ve 120. saatlerde jugular venapunktur yöntemi ile toplandı. Kan örnekleri hematolojik ve biyokimyasal parametreleri belirlemek için analiz edildi. Sefkuinomun tekrarlanan subkutan uygulaması hematolojik ve biyokimyasal parametrelerde herhangi bir değişikliğe neden olmadı ($P > 0,05$). Bu sonuçlar, koyunlarda 2,5 mg/kg dozda sefkuinomun günde bir defa beş gün boyunca tekrarlanan subkutan uygulamasının güvenli ve iyi tolere edilebileceğini gösterdi. Ancak, koyunlarda farklı dozlarda ve uygulama yollarında tekrarlanan uygulamadan sonra sefkuinomun güvenilirliğinin moleküler ve histopatolojik olarak araştırılmasına ihtiyaç vardır.
Anahtar kelimeler: Biyokimya, sefkuinom, hematoloji, koyun.

Introduction

Cefquinome is a fourth-generation cephalosporin antibiotic developed exclusively for use in animals (CVMP, 2003). Cefquinome has a broad spectrum of antibacterial activity, including gram-negative and gram-positive bacteria, and it is considered to be highly stable against β-lactamases encoded by chromosomes and genes on plasmids (Durckheimer et al., 1988; Limbert et al., 1991). The main difference between cefquinome and previous generations is that it has a zwitterionic molecular structure, which increases its ability to penetrate the periplasmic space of the bacterium and improves resistance to β-lactamases (Limbert et al., 1991). In addition, cefquinome has favorable pharmacokinetic properties such as good absorption, high bioavailability, and primary elimination via the kidney (Corum et al., 2019). It has been approved for the treatment of acute mastitis and foot rot in cattle, respiratory tract diseases in cattle, pigs, and horses, calf and foal septicemia, and metritis-mastitis-agalactia syndrome in sows (CVMP, 1995; 1999; 2003).

Due to its broad antibacterial spectrum and excellent efficacy, cefquinome is used as an extra label in sheep for purposes indicated in other species. For the effective and safe use of drugs, the undesirable effects of extra-label use should be evaluated. Hematological and biochemical parameters may be useful in evaluating the adverse effects of drugs. Hematological parameters are used to evaluate bone-marrow functions and biochemical parameters are used to evaluate the functions of organs such as the liver and kidney (Corum et al., 2015; Corum et al., 2016; Durna Corum et al., 2020). Although the effect of cefquinome on hematological and biochemical parameters after repeated (2 mg/kg, every 24 h for 5 days) IM administration in sheep was revealed, no information was found after repeated SC administration. The aim of this study is to determine the effect of repeated (every 24 h for 5 days) subcutaneous administration of cefquinome at a dose of 2.5 mg/kg on hematological and biochemical parameters in sheep.

Material and Methods

***Animals*:** Six healthy female Merinos sheep, aged 1.5 ± 0.2 years and weighing 39 ± 2.7 kg, were used. The animals were judged as healthy by a physical examination, and they had not received any drug during the 4 weeks prior to the study. The sheep were kept in individual pens for 2 weeks before the study for the acclimation period. The sheep were fed with drug-free commercial feed twice a day, and alfalfa hay and water were given ad libitum.

***Experimental design*:** Cefquinome (Cobactan 2.5%, Intervet, İstanbul/Turkiye) was administered subcutaneously at a dose of 2.5 mg/kg into the axillary region of each sheep once daily for five consecutive days. Blood samples were collected into gel-containing tubes

for biochemical analyses (2 mL) and into EDTA-containing tubes for hematological analyses (2 mL) from the jugular vein through jugular venipuncture prior to drug administration (0 h) and at 48 h and 120 h following the first drug administration. For the analysis of biochemical parameters, blood samples were centrifuged at 4000 g for 10 min, and the separated serum was stored at −80°C until analysis. Additionally, sheep were observed clinically during the study.

***Hematological and biochemical analyzes*:** Hematological parameters such as white blood cell (WBC), red blood cell (RBC), hemoglobin, hematocrit, and platelet were measured by hemocell counter (Shenzhen Mindray Bio-Medical Electronics, BC-2800 Auto Hematology Analyzer, China). Biochemical parameters such as albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), cholesterol, creatine kinase (CK), creatinine, gamma-glutamyltransferase (GGT), total protein (TP), and triglyceride were analyzed by autoanalyzer (ILab-300 bioMerieux Diagnostics, Milan, Italy).

***Statistical analysis*:** All values were presented as mean ± SD. The normality of the data distribution was assessed with the *Shapiro-Wilk* test and the homogeneity of variance with *Levene's* test. Hematological and biochemical results were analyzed using a one-way analysis of variance and post hoc *Tukey* tests. SPSS 22.0 (IBM Corp, Armonk, NY) statistics program was used for the statistical analysis. $P < 0.05$ value was considered statistically significant.

Results

No local (lameness, redness, and pain at the injection site) or systemic (feed and water consumption, fecal production, and behavior) adverse effects were observed in any sheep after repeated administration of cefquinome. Hematological and biochemical parameters in sheep after repeated subcutaneous administration of cefquinome (2.5 mg/kg, every 24 h for 5 days) are presented in Table 1 and Table 2, respectively. Hematological (WBC, RBC, hemoglobin, hematocrit, and platelet) and biochemical (albumin, ALP, ALT, AST, BUN, cholesterol, CK, creatinine, GGT, TP, and triglyceride) parameters did not alter following repeated subcutaneous administration of cefquinome ($P > 0.05$).

Table 1: Effect of cefquinome (2.5 mg/kg, subcutaneous, every 24 h for 5 days) in sheep on hematological parameters (n = 6, mean ± SD)

Parameters	0 h	48 h	120 h
WBC ($^{x}10^{9}$/L)	7.67 ± 1.16	7.17 ± 1.13	6.97 ± 0.67
RBC ($^{x}10^{12}$/L)	12.73 ± 0.65	12.00 ± 1.31	11.95 ± 1.25
Hemoglobin (g/dL)	11.83 ± 1.01	10.95 ± 0.85	11.48 ± 1.04
HCT (%)	34.57 ± 2.17	32.02 ± 3.05	31.35 ± 2.78
Platelet ($^{x}10^{9}$/L)	301.33 ± 76.66	304.17 ± 72.46	269.17±55.95

WBC; white blood cells, RBC; red blood cells, HCT; hematocrit.

Table 2: Effect of cefquinome (2.5 mg/kg, subcutaneous, every 24 h for 5 days) in sheep on biochemical parameters (n = 6, mean ± SD)

Parameters	0 h	48 h	120 h
ALB (g/dL)	2.97 ± 0.18	2.98 ± 0.29	2.98 ± 0.18
ALP (U/L)	58.83 ± 14.50	65.17 ± 21.14	68.00 ± 23.26
ALT (U/L)	26.00 ± 7.72	26.67 ± 7.28	28.17 ± 9.17
AST (U/L)	94.17 ± 11.62	96.83 ± 14.27	95.83 ± 9.97
BUN (mg/dL)	22.97 ± 2.87	20.87 ± 4.43	20.30 ± 2.89
CHOL (mg/dL)	67.50 ± 9.57	66.67 ± 11.34	62.17 ± 10.48
CK (U/L)	205.83 ± 23.64	181.33 ± 28.16	190.33 ± 34.99
CREAT (mg/dL)	0.60 ± 0.03	0.57 ± 0.08	0.52 ± 0.06
GGT (U/L)	47.00 ± 13.87	46.67 ± 13.06	47.33 ± 13.74
TP (g/dL)	6.63 ± 0.44	6.66 ± 0.68	6.58 ± 0.24
TG (mg/dL)	15.50 ± 3.56	18.33 ± 3.39	17.83 ± 2.93

ALB; albumin, ALP; alkaline phosphatase, ALT; alanine aminotransferase, AST; aspartate aminotransferase, BUN; blood urea nitrogen, CHOL; cholesterol, CK; creatine kinase, CREAT; creatinine, GGT; gamma-glutamyltransferase, TP; total protein, TG; triglyceride.

Discussion

Drugs may cause adverse side effects. The side effects can be classified as pharmacological, biochemical, pathological, genotoxic, and allergic reactions. Hematological and biochemical parameters are used to evaluate the effects of drugs on physiological and pathological conditions (Maden et al., 2001; Altan et al., 2019). Hematological parameters (WBC, RBC, hemogram, hematocrit, platelet) reflect bone-marrow functions and fluid-electrolyte balance situations. Biochemical parameters (albumin, ALP, ALT, AST, BUN, cholesterol, CK, creatinine, GGT, TP, and triglyceride) reflect liver, kidney, muscle, and lipid

metabolism function (Turgut, 2000; Kerr, 2002a; Kerr, 2002b). Cephalosporins have a high therapeutic index and a very low incidence of adverse drug reactions. The adverse effects of cephalosporins, except for hypersensitivity, depend on the dose and duration of administration. Depending on the dose and duration of administration, cephalosporins cause neutropenia, thrombocytopenia, agranulocytosis, glomerular and interstitial nephritis, tubular necrosis, hepatitis, and neurotoxicity (Caprile, 1988; Maden et al., 2001).

In this study, the repeated administration of cefquinome via the subcutaneous route in sheep did not cause any change in hematological values. It has been reported that the IM administration of cefquinome to sheep (2 mg/kg, every 24 h for 5 days, Rana et al., 2015) and dogs (1 mg/kg, every 24 h for 14 days, Maden et al., 2001) causes no significant changes in hematological parameters. While the repeated (intravenous, every 12 h for 7 days) administration of cefquinome to horses at a dose of 1-6 mg/kg did not cause any change in WBC and platelet, the changes in RBC, hemoglobin, and hematocrit values were reported within reference values (Altan et al., 2019). In addition, the repeated (IM, 2 mg/kg, every 24 h for 7 days) administration of cefquinome in buffalo calves changed the hemoglobin value (Mangal et al., 2015).

In this study, the repeated administration of cefquinome subcutaneously in sheep did not cause any change in biochemical parameters. It has been reported that the repeated administration of cefquinome did not cause any change; in the values of ALT, AST, ALP, TP, albumin, glucose, and total bilirubin in the dogs (Maden et al., 2001); in the values of albumin, ALP, ALT, AST, cholesterol, creatinine, GGT, lactate dehydrogenase, total bilirubin, and TP in the horse (Altan et al., 2019); in the values of AST, ALT, ALP, urea, creatinine, albumin, and TP in the camels (Kant et al., 2019). While the repeated (2 mg/kg, IM, every 24 h for 7 days) administration of cefquinome did not cause any change in ALT, ALP, AST, and GGT activities in buffalo calves, it changed BUN and creatinine values (Mangal et al., 2015).

Conclusion

This study showed that subcutaneous administration of cefquinome at a dose of 2.5 mg/kg every 24 hours for 5 days did not affect hematological and biochemical parameters in sheep. However, there is a need for molecular and histopathological investigation of the safety of cefquinome after repeated administration in sheep at different doses and administration routes.

Financial Support

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Ethical Statement

The experiment was approved (2015/43) by the Ethics Committee of the Faculty of Veterinary Medicine of Şelcuk University (Konya/Turkiye) and carried out in accordance with the European Directive (2010/63/EU).

Conflict of Interest

The authors declared that there is no conflict of interest.

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Investigation of tap water quality of Bingöl University

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Abstract: In this research, the chemical and microbiological quality of 20 tap water samples were randomly selected from Bingöl University in Bingöl. The Heterotrophic Plate Counts (HPC) numbers of samples were 1.09 ± 0.4 logs CFU/ml. The coliform and fecal coliform bacteria were not detected in all samples. The average pH value of samples was 6.79 ± 0.15 and all samples were nitrate negative, nitrate positive, qualitatively. The average hardness values of the water samples were determined as 6.16 ± 2.47 ºf. It can be said that all samples are classified as soft water. As a result, it is thought that tap water's simple chemical and microbiological quality in Bingöl University is sufficient for drinkability, However, this thesis should be supported by forwarding microbiological, chemical, and sensory analyses.
Keywords: Tap water, microbiological quality, chemical quality

Bingöl Üniversitesi musluk sularının kalitesinin araştırılması

Özet: Bu araştırmada Bingöl ilinde bulunan Bingöl Üniversitesindeki rastgele seçilmiş 20 musluk suyundan alınan örneklerin mikrobiyolojik ve kimyasal kalitesi araştırılmıştır. Örneklerdeki ortalama Heterotrophic Plate Counts (HPC) sayısı 1,09 ± 0,4 log CFU/ml olarak tespit edilmiştir. Örneklerin hiçbirinde koliform ve fekal koliform grubu bakteri tespit edilmemiştir. Kimyasal olarak örneklerdeki ortalama pH ve sertlik değerleri sırasıyla 6,79 ± 0,15 ve 6,16 ± 2,47 ºf olarak tespit edilmiştir. Ayrıca, örneklere kalitatif olarak nitrat ve nitrit testleri uygulanmış ve tümü nitrat pozitif, nitrit negatif bulunmuştur. Su numunelerinin ortalama sertlik değerleri ise 6,16 ± 2,47 ºf olarak belirlenmiştir. Tüm örneklerin yumuşak sular sınıfına girdiği söylenebilir. Sonuç olarak Bingöl Üniversitesi'ndeki musluk suyunun basit kimyasal ve mikrobiyolojik kalitesinin içilebilirlik için yeterli olduğu fakat bu tezin ileri mikrobiyolojik, kimyasal ve duyusal analizlere desteklenmesi gerektiği düşünülmektedir.
Anahtar Kelimeler: Musluk suyu, mikrobiyolojik kalite, kimyasal kalite

Introduction

Is known that drinking water may cause diseases or spread diseases. Continuously reaching for healthy and safe drinking water is a basic principle for protecting public health (Tuluk & Orhan, 2017). Reaching healthy water is a human’s right. For this reason, drinking water services are supplied by the government in Turkey. Healthy and safe water is supplied by

local authorities and it is monitored by the Ministry of Health (Koksal & Samastı, 2007). In most cases, drinking water requirements for city folk are met by the network water obtained by treatment (precipitation, filtration, purification, disinfection) of surface waters (Tuluk & Orhan, 2017).

Perception of tap water is subject to a wide range of factors and interactions. Despite the access to drinkable tap water quality in developing countries supplied at cost-efficient, the consumption of bottled water has increased over the past years. One of the main reasons for this increase is the perception among citizens that tap water is unhealthier and tastes worse than bottled water. The water consumption profile is strongly linked with user satisfaction (taste, odor, color) (Delpla et al., 2020). On the other hand, researchers showed a positive correlation between tap water consumption and a water quality index assembly information on water quality parameters such as turbidity, color, free chlorine residual, and heterotrophic plate counts (Proulx et al., 2010). In general, users do not know about the daily use of tap water quality. In the light of this information, this study aims to investigate the chemical and microbiological quality of tap water in the university which, people daily use.

Materials and Methods

Microbiological Analyses: Turkish Standards Institute, TS-266 directives were followed while collecting tap water samples (Türk Standartları Enstitüsü, 2005). Five samples were collected on each analysis day and it took a month to collect 20 samples in total. Tap water samples were collected into sterile 200 ml glass bottles from the faculty buildings in the university for microbiological and chemical analysis. The samples were immediately transported microbiology laboratory. The standard plate count technique was used to enumerate heterotrophic culturable bacteria in water (Harrigan, 1998). Plates incubate at 37 ℃ for 24-48 h. The average number of colonies was calculated as log CFU/ ml.

For enumeration of Coliform and fecal coliform bacteria, the samples were subjected most probable number (MPN) test following procedures Food and Drug Administration's Bacteriological Analytical Manual (Çakır et al., 2002).

Chemical Analyses: When microbiological analyses were completed, pH analysis was performed using a pH meter (PH Selecta, pH 2001) (AOAC, 1984). For qualitative nitrite and nitrate analyses, the method described by Tolgay & Tetik (1964) was used. For the determination of French hardness, the method previously described by Tolgay & Tetik (1964) was used. The results were explicated according to Table 1.

Results

All microbiological and chemical data are shown in Table 1. The HPC, pH, and Hardness results were expressed as average ± standard deviation (X ± SD). Other qualitative results were expressed as negative or positive.

Table 1: Microbiological and chemical data of collected water samples in the University region.

Microbiological	HPC (log_{10} CFU/ml)	Total coliform	Fecal coliform	
	1.09 ± 0.4	Negative	Negative	
Chemical	pH	Total Hardness	Nitrite	Nitrate
	6.79 ± 0.15	6.16 ± 2.47 ºf	Negative	Positive

Discussion

The microbial quality of tap water samples in this study was not of concern. HPC was not detected in 60% (12) of the samples. An average of 1.09 ± 0.4 log CFU/ml microorganisms were detected in the remaining 40% (8) samples (Table 1). Microorganisms will usually grow in water, and on surfaces in contact with water as biofilms. Growth following drinking water treatment is normally referred to as 'regrowth'. The principal determinants of regrowth are temperature, availability of nutrients (organic matter), and lack of residual disinfectant (etc. Chlorine) (Robertson & Brooks 2003). Koçak & Güner (2009) reported an average of 3.88 ± 3.68 log CFU/ml HPC numbers in 45 tap water samples in Erzurum. This value is considerably higher than our results. Control of fecal contamination in drinking water systems and sources where it occurs is of primary concern. Fecal-specific indicator bacteria such as *Escherichia coli* (*E. coli*) are the parameters of primary importance in monitoring fecal pollution (Robertson & Brooks, 2003). Coliform and fecal coliform were not detected in any of the tap water samples in this study (Table 1). These results are consistent with most of the studies on tap water quality (Can, 2000; Alim, 1995). Although Kocak & Güner (2009) isolated coliform group microorganisms from well water and reservoir water samples, this microorganism group was not found in any tap water samples. The microbiological data in this study showed that the treatment and monitoring of the university mains waters are well.

pH is clearly an important water quality parameter. If pH values are higher than 8, water is unsuitable for effective disinfection and, this situation can cause a slippery feeling. Also, the value of less than 6.5 of water can have a corrosive and metallic taste (Tuluk & Orhan, 2017).

In this study, the average pH value of samples is 6.79 ± 0.15 and all samples have acceptable pH values.

As expected, nitrate was detected in all of the samples, while nitrite could not be detected qualitatively in any of the samples. It is known that nitrate in drinking water is an important risk factor for methemoglobinemia in bottle-fed infants (WHO, 2011). Nitrate levels in this study are uncertain. Further analysis is required in terms of question marks. In the drinking water regulation of the Ministry of Health, it is stated that nitrate in drinking water should not exceed 50 mg/l (Resmi Gazete, 2005).

Table 2: Water hardness (total hardness) scale

Water Hardness	Soft	Average	Hard	Very Hard
French Hardness (ºf)*	0-10	11-20	21-30	> 30

*1 ºf = 10 mg/L as $CaCO_3$

The average hardness values of the water samples were determined as 6.16 ± 2.47 ºf (Table 1). When the samples are evaluated according to Table 2, it can be said that all samples are classified as soft water. Similarly, Can (2000) reported that the total hardness value of tap water used as drinking water in the Balıkesir region is between 8.21-10.53 °f. Bigin (2003) determined the hardness value between 12.3 and 16.8 °f in water samples taken from different points of the drinking water network of Niğde Province. These results show that the tap waters in the Niğde are of average hardness class (Table 2). In a study conducted in Erzurum, the hardness rate was 7.96 (Kocak & Güner, 2009). Our results are consistent as the Bingöl and Erzurum regions are geographically close to each other.

Conclusions

As a result, it is thought that the simple chemical and microbiological quality of tap water in Bingöl University is sufficient for drinkability, However, this thesis should be supported by forwarding microbiological, chemical, and sensory analyses. Generally, drinking water quality parameter values of the cities are regularly published on the Water and Sewerage Administration website of that city. Although the competent authority in Bingöl does not have an official website, it is recommended that it be in the future.

Financial Support

This research received no grant from any funding agency/sector.

Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

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Evaluation of bacteria isolated from different animal species and antibiotic resistance in the veterinary diagnostic laboratory

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Abstract: Isolation of bacteria can be performed by taking correct and convenient samples, especially from the infection site. In addition, after isolation of the bacteria, the antimicrobial susceptibility test should be performed routinely to treat animals conveniently. For this purpose, 129 isolates were included in the current study from different animal origins and different examination materials in the culture collection of Atatürk University Faculty of Veterinary Medicine Department of Microbiology between 2020 and 2021. The isolates were identified by bacteriological methods and their antibiotic resistance was evaluated phenotypically. In the study, it was determined that bacteria belonging to the *Staphylococcus* genus (27.2%) were mostly involved in different infections. Overall results displayed those bacteria tested in this study were resistant to neomycin (100%), penicillin (76.74%), oxytetracycline (73.80%), and sulfamethoxazole-trimethoprim (61.25%) with a different rate, whereas they were susceptible to cephalosporin antibiotics (cefovecin %64.3 ceftiofur %80, and cefoxitin %81.8) used in the current study.
Keywords: Antimicrobial resistance, bacteria, fungi, retrospective study

Veteriner tanı laboratuvarında farkli hayvan türlerinden izole edilen bakterilerin ve antibiyotik direnç durumlarinin değerlendirilmesi

Özet: Özellikle enfeksiyon bölgesinden doğru ve uygun örnekler alınarak mikroorganizma izolasyonu yapılabilir. Ek olarak, bakterilerin izolasyonundan sonra, hayvanı uygun şekilde tedavi etmek için antimikrobiyal duyarlılık testi rutin olarak yapılmalıdır. Bu amaçla Atatürk Üniversitesi Veteriner Fakültesi Mikrobiyoloji Anabilim Dalı kültür koleksiyonunda, 2020-2021 yılları arasında farklı hayvan türleri ve farklı inceleme materyallerinden elde edilen 129 izolat mevcut çalışmaya dahil edildi. İzolatlar uygun yöntemlerle identifiye edilerek, fenotipik olarak antibiyotik dirençlilikleri değerlendirilmiştir. Çalışmada, en fazla *Staphylococcus* cinsine ait bakterilerin (%27,2) farklı infeksiyonlarda rol aldığı saptanmıştır. Genel sonuçlar, bu çalışmada test edilen bakterilerin farklı oranlarda neomisin (%100), penisilin (%76,74), oksitetrasiklin (%73,80) ve sülfametoksazol trimetoprim'e (%61,25) dirençli olduğunu, oysa ki bu çalışmada kullanılan sefalosporin antibiyotiklerine (sefovesin %64,3, seftiofur %80 ve sefoksitin %81,8) duyarlı olduklarını gösterdi.
Anahtar Sözcükler: Antimikrobiyal direnç, bakteri, mantar, retrospektif çalişma

Introduction

Infectious diseases caused by different microorganisms provoke various problems in animals, especially yield losses. Bacteria constitute most of these microorganisms. These bacterial infections are characterized by different clinical findings. Fungi, as well as bacteria, can cause infections in animals. To determine the treatment protocols of infections, it is necessary to determine the agent and, if the infection is caused by bacteria, these protocols should be arranged by using antibiotic susceptibility tests (EUCAST, 2017).

Bioactive substances that act on bacteria in different ways, preventing their development and growth or killing bacteria are called antibiotics. The ability of a bacterium to resist the lethal and inhibitory effect of antibiotics is called antibiotic resistance. Although this resistance can be found structurally in bacteria, it can also be acquired later (Denyer et al., 2004). Wrong choice of antibiotics as a result of misdiagnosis of physicians and widespread, unconscious, and continuous use of antibiotics play a role in the development of resistance in bacteria as well as take in place different mechanisms under developing antimicrobial resistances (Barnes et al., 2013).

Antibiotic resistance gained by bacteria is becoming a global threat. Because it limits the possibilities of drugs for the treatment of infections, increases treatment costs, and causes animal and loss of productivity. Adding antibiotics to animal feeds to accelerate growth and prevent disease formation creates a public health problem by causing the transfer of antibiotic-resistant bacteria found in animal foods to humans (Kilic, 2004). Various antibiotic susceptibility tests are performed in routine diagnostic laboratories to determine the antibiotic resistance status of the bacteria and to select the antibiotics to be used in the treatment more accurately (CLSI, 2017). In this study, bacteria isolated from different tissues and organs of different animal species and their antibiotic resistance status were evaluated.

Material and Method

In this research, 129 isolates belonging to the year 2020-2021, which were previously recorded in the culture collection of Ataturk University, Faculty of Veterinary Medicine, Department of Microbiology, were used. Microorganisms were isolated from different samples (skin lesion, urine, feces, blood, milk, tissue samples (lung, liver, spleen, heart, kidney), eye, ear, wound, joint, stomach content, mucous membranes, oral, nasal, pharyngeal, and rectal swabs) of various animal species (large ruminant, small ruminant, pet animals, poultry, horse, and rabbit) were recovered from -80 °C and *Gram* staining and biochemical tests were performed. Blood agar (Merck Cat No: 1.10886.0500, Germany), MacConkey agar

(Merck Cat No: 1.05465.0500, Germany), and Nutrient agar (Merck Cat No: 1.05450.0500, Germany) were used during the passage. Moreover, Mueller Hinton agar media (Merck Cat No: 1.05437.0500, Germany) was used for the antibiotic susceptibility test and Mycoplasma Agar Base (Oxoid Cat No: CM0401, United Kingdom) was used for the identification of *Mycoplasma* spp. catalase, oxidase, coagulase, biochemical tests (carbohydrate fermentation tests, motility test, urease activity, nitrate reduction test, hydrogen sulfide production test, ONPG test, gelatin hydrolysis test, etc.) were performed for the identification of bacterial agents (Quinn, 2004).

Disc diffusion technique was used to determine the antibiotic resistance profile of bacteria. The *Gram* characteristic of the isolates, as well as the animal and sample type from which the bacteria were isolated, were considered when selecting antibiotic discs. Inhibition zone diameters formed because of the test were measured and compared with the specified standards and antibiotics to which the identified bacteria were susceptible and resistant were determined (EUCAST, 2017).

Sabouraud Dextrose Agar (SDA) (Merck Cat No: 1.07315.0500, Germany) was used for mycological examination. After the isolates were inoculated on an SDA medium, after 21 days of incubation at 25 °C, fungal colonies were stained with Lactophenol cotton blue (Merck, Cat No: 113741, Germany), and fungal species were identified (Campbell et al., 2013). SDA containing 1.0% olive oil was used for suspected yeast isolates. After 7-10 days of incubation at 37 °C, the growing colonies were stained by the *Gram* staining method and identified by urease production and melanin production (Larone, 2002; Quinn, 2004). All obtained data were reported and recorded, and isolates were grouped according to animal species.

Results

Seven fungi and seven yeasts were identified among the 129 microorganisms in the culture collection, while 26.95% of the 115 bacteria were *Staphylococcus* spp., 33.91% were *Enterobacteriaceae*, 13.91% were *Pasteurellaceae*, 9.56% were *Actinomyces* sp., 7.82% were *Streptococcus* sp., 4.34% were *Enterococcus* sp., 1.73% were *Alcaligenes* sp. The bacteria membered of *Enterobacteriaceae* were isolated from cattle, calves, and poultry. In addition, most of *Staphylococcus* spp. were isolated from cats and dogs, whereas *Actinomycetaceae* was detected in sheep, goats, and lambs. When all animal species and isolates were evaluated, it was observed that the majority belong to the *Staphylococcus* spp. (27.2%), followed by the

families *Enterobacteriaceae* (25.5%) and *Pasteurellaceae* (14%). Figure 1 depicted the distribution of bacteria according to animal origins.

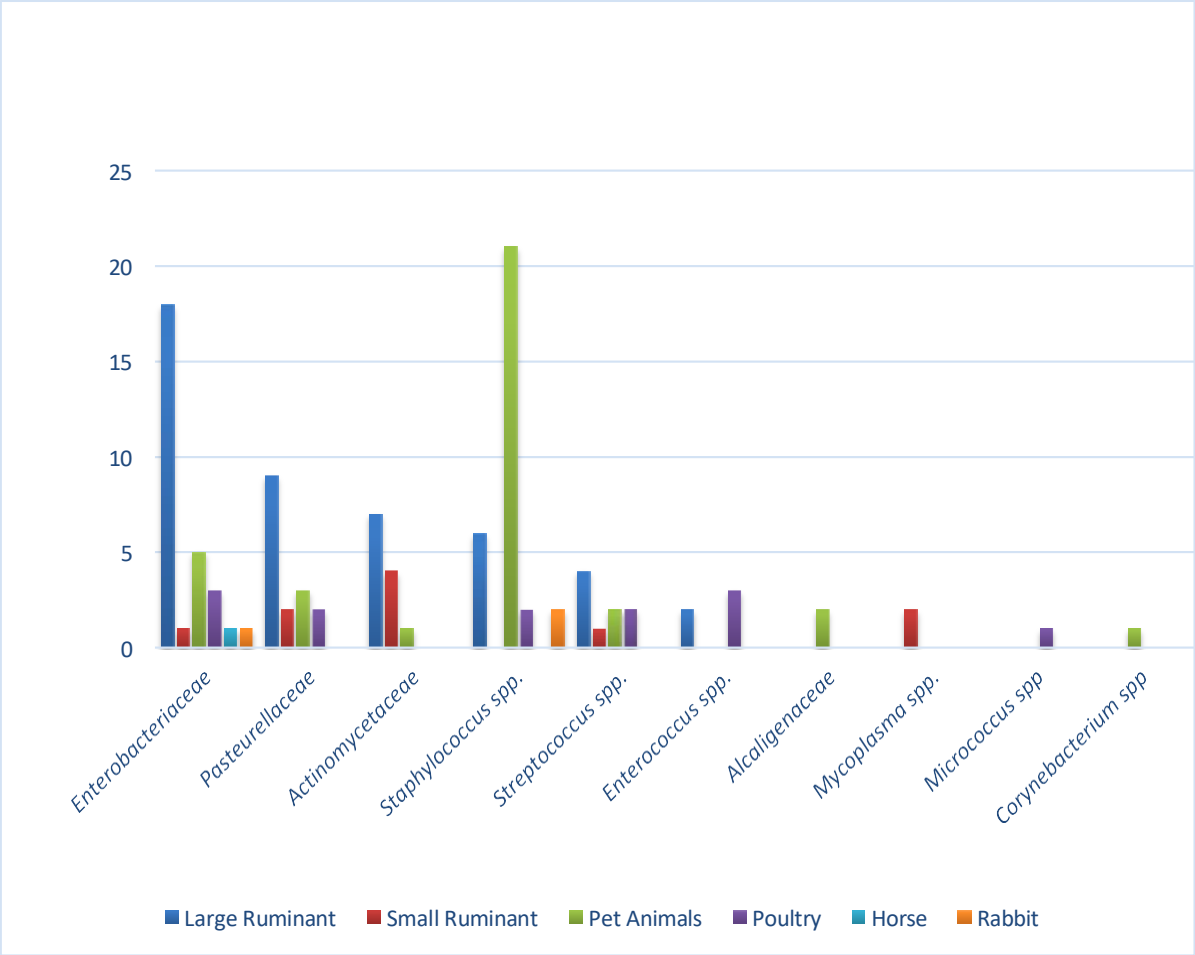

Figure 1: Distribution of identified bacterial families and general by animal origins

The origin of the isolates including bacteria and fungi were large ruminants (six abscesses, one skin lesion, six tissue samples, two wounds, one ear, one nasal, three fecal, and twelve joint swabs), horse (one skin lesion and abscess), small ruminants (nine tissue samples), pet animals (six skin lesion, three urine, one blood, four milk, one tissue sample, six eyes, four ears, two wounds, one oral, one nasal, one pharyngeal, and one discharge from the mucous membranes swabs), poultry (one tissue samples, one stomach contents, twelve fecal, one eye, and one laryngeal swab), and rabbit (one abscess and rectal swab). The distribution of bacterial families isolated from different sample types was shown in Table 1.

Table 1: Distribution of families of bacteria isolated from different sample groups (n)

	Enterobacteriaceae	*Pasteurellaceae*	*Actinomycetaceae*	*Staphylococcus* spp.	*Streptococcus* spp.	*Enterococcus* spp.	*Alcaligenaceae*	*Mycoplasma* spp.	*Micrococcus* spp.	*Corynebacterium* spp.
Abscess	5	0	3	3	2	1	0	0	0	0
Nasal swab	3	1	1	2	0	0	1	0	0	0
Skin lesion	1	0	0	2	0	0	0	0	0	0
Feces	7	0	0	1	2	3	0	0	0	0
Joint swab	4	6	2	2	2	0	0	0	0	0
Ear swab	0	0	0	5	0	0	0	0	0	0
Tissue samples	4	6	5	1	2	1	0	2	0	0
Rectal swab	1	0	0	0	0	0	0	0	0	0
Urine	1	0	0	1	0	0	1	0	0	0
Blood	0	0	0	0	0	0	0	0	0	1
Wound swab	2	1	0	2	0	0	0	0	0	0
Mucous membrane	0	0	0	0	1	0	0	0	0	0
Oral swab	0	0	0	1	0	0	0	0	0	0
Milk	0	0	0	4	0	0	0	0	0	0
Pharyngeal swab	1	1	0	0	0	0	0	0	0	0
Eye swab	0	0	0	6	0	0	0	0	1	0
Stomach contents	0	1	0	1	0	0	0	0	0	0

The seven isolated fungal agents were identified as *Aspergillus fumigatus* (n=2), *Blastomyces dermatitis* (n = 1), *Penicillium* spp. (n = 2), *Purpureocilium lilacium* (n = 1), and *Mucor* spp. (n = 1). All fungi were isolated from horse (n = 1), cattle (n = 2), and pet animals (n = 4) with skin lesions. A total of seven yeast were identified as *Macrorhabdus ornitogaster*

(85.72%) and *Candida* spp. (14.28%). Although *Macrorhabdus ornitogaster* was isolated from the fecal samples of different ornamental birds, *Candida* spp. was isolated from the laryngeal swab of the peacock.

When the antibiogram results of the bacteria whose identification was completed were evaluated, high resistance to (75%) trimethoprim-sulfamethoxazole was observed in bacteria belonging to the *Enterobacteriaceae* family, while low resistance to (20%) ceftiofur and (18.18%) cefoxitin was observed. *Pasteurellaceae* were found to be highly resistant to (35.29%) aminoglycoside group antibiotics, while low resistance to (8.33%) ampicillin-sulbactam was detected. *Staphylococcus* spp. were also found to be resistant to the (75%) tetracycline group, even though the low resistance to (8.69%) ceftiofur was found. *Actinomycetaceae* spp. were founded to be resistant to (100%) trimethoprim-sulfamethoxazole, (100%) tetracycline, (83.33%) enrofloxacin, and (72.72%) gentamicin, however, these bacteria were susceptible to ceftiofur. Gentamicin resistance was observed in 85.71% of bacteria belonging to the genus *Streptococcus* spp., while resistance to ceftiofur was detected at a rate of 14.28%. The distribution of antibiotic resistance bacterial families was represented in Table 2.

Table 2: Antibiotic resistance distribution of isolated bacterial families

Antibiotics*	E*nterobacteriaceae*		*Pasteurellaceae*		*Staphylococcus* spp.		*Actinomycetaceae*		*Streptococcus* spp.		*Enterococcus* spp.		*Alcaligenaceae*	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Gentamicin	9 (36)	16 (64)	5 (38.5)	8 (61.5)	9 (32.1)	19 (67.9)	8 (72.7)	3 (27.3)	6 (85.7)	1 (14.3)	3 (75)	1 (25)	0 (0)	2 (100)
Marbofloxacin	12 (48)	13 (52)	2 (15.4)	11 (84.6)	7 (25.9)	20 (74.1)	5 (45.5)	6 (54.5)	2 (40)	3 (60)	3 (75)	1 (25)	1 (50)	1 (50)
Enrofloxacin	12 (57.1)	9 (42.9)	4 (33.3)	8 (66.7)	5 (27.8)	13 (72.2)	5 (83.3)	1 (16.7)	1 (33.3)	2 (66.7)	3 (75)	1 (25)	1 (50)	1 (50)
Tetracycline	8 (100)	0 (0)	2 (50)	2 (50)	14 (66.7)	7 (33.3)	4 (100)	0 (0)	2 (66.7)	1 (33.3)	1 (100)	0 (0)	0 (0)	1 (100)
Ciprofloxacin	9 (45)	11 (55)	2 (20)	8 (80)	9 (34.6)	17 (65.4)	5 (55.6)	4 (44.4)	2 (40)	3 (60)	0 (0)	0 (0)	1 (50)	1 (50)
Amoxicillin Clavulanic Acid	13 (48.1)	14 (51.9)	2 (14.3)	12 (85.7)	5 (17.2)	24 (82.8)	2 (20)	8 (0)	4 (50)	4 (50)	2 (50)	2 (50)	0 (0)	2 (100)
Ampicillin Sulbactam	10 (40)	15 (60)	1 (8.3)	11 (91.7)	4 (17.4)	19 (82.6)	2 (18.2)	9 (81.8)	2 (28.6)	5 (71.4)	2 (50)	2 (50)	0 (0)	1 (100)
Trimethoprim-Sulfamethoxazole	18 (75)	6 (25)	3 (33.3)	6 (66.7)	11 (40.7)	16 (59.3)	8 (100)	0 (0)	4 (66.7)	2 (33.3)	4 (100)	0 (0)	1 (50)	1 (50)
Cefoxitin	4 (18.2)	18 (81.8)	1 (9.09)	10 (90.9)	5 (20)	20 (80)	1 (10)	9 (90)	0 (0)	4 (100)	2 (100)	0 (0)	1 (50)	1 (50)
Cefovecin	5 (35.7)	9 (64.3)	1 (20)	4 (80)	1 (9.1)	10 (90.9)	1 (20)	4 (80)	2 (66.7)	1 (33.3)	1 (50)	1 (50)	0 (0)	1 (100)
Ceftiofur	5 (20)	20 (80)	2 (15.38)	11 (84.6)	2 (8.7)	21 (91.3)	0 (0)	11 (100)	1 (14.3)	6 (85.7)	0 (0)	3 (100)	1 (50)	1 (50)
Tobramycin	3 (50)	3 (50)	0 (0)	3 (100)	4 (28.6)	10 (71.4)	2 (100)	0 (0)	2 (100)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)
Oxytetracycline	12 (80)	3 (20)	2 (28.57)	5 (71.4)	7 (100)	0 (0)	5 (71.4)	2 (28.6)	4 (100)	0 (0)	2 (100)	0 (0)	1 (100)	0 (0)
Neomycin	5 (100)	0 (0)	1 (100)	0 (0)	6 (100)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)
Spiramycin	3 (100)	0 (0)	0 (0)	1 (100)	0 (0)	3 (100)	1 (100)	0 (00)	0 (0)	0 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Penicillin	9 (100)	0 (0)	2 (66.7)	1 (33.3)	9 (90)	1 (10)	1 (25)	3 (75)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

*: The antibiotics were selected according to the sample types. Hence, some of the data missing for some isolates. R: Resistant, S: Susceptible

Discussion

The bacterial and fungal agents are routinely isolated in the diagnostic laboratory (Nocera et al., 2021; Kakooza et al., 2021; Jonker and Michel, 2021). Those studies have indicated not only single animal results but also antimicrobial resistance results of all samples from different origins. These reports are clearly essential to understanding and revealing antimicrobial resistance over the years. In this regard, the current study reports bacterial and fungal agent results isolated from different animal origins in the veterinary diagnostic laboratory between 2020 and 2021.

A study reported that the most common bacteria were coagulase-negative staphylococci (CNS) from ear samples of cats (Nocera et al., 2021). Similarly, CNS were isolated and identified from all ear samples in the current study. The antimicrobial resistance of CNS isolates was reported to be 64% against amoxicillin-clavulanic acid (Nocera et al., 2021). However, 17% of CNS in the current study was found to be resistant to amoxicillin-clavulanic acid. *Trueperella pyogenes* (*T. pyogenes*) were isolated from different clinical samples collected from cattle, sheep, goats, pigs, horses, dogs, and buffaloes (Ribeiro et al., 2015). The most common sample types of isolated *T. pyogenes* were reported as the mammary gland, abscess, and tissue samples of affected animals in the same study. In addition, those *T. pyogenes* isolates were highly resistant to (9.2%) tetracycline and (49.3%) trimethoprim-sulfamethoxazole (Ribeiro et al., 2015). It has been reported that the resistance rate of *T. pyogenes* isolated from uterine samples of cattle was 91.8% against trimethoprim-sulfamethoxazole (Adiguzel et al., 2021). Another study reported that *Escherichia coli* (*E. coli*) and *Klebsiella* spp. were isolated from the samples containing mastitis, wound, otitis, urinary tract, and respiratory tract samples from different animal origins. In the same study, *E. coli* was isolated from mastitis samples mostly, whereas *Klebsiella* spp. was isolated from wound swabs (Puvarajan et al., 2020). In contrast, *Pasteurella* spp. and *Staphylococcus* spp. isolated from wound swabs mostly, whereas *Staphylococcus* spp. was isolated from mastitis cases in the current study. In the same study, *Pseudomonas* spp. was isolated from urinary tract samples (Puvarajan et al., 2020), and even though *Alcaligenes* spp., methicillin-resistant *Staphylococcus felis*, and *E. coli* were isolated in the same samples in the current study. On the other hand, the antimicrobial resistance of *Staphylococcus* spp. isolated from tissue and joint samples were detected against (93.28%) tetracycline and (91.7%) penicillin (Puvarajan et al., 2020), which is similar to the current study results.

A study reported that 17 coagulase-positive staphylococci, two beta-hemolytic streptococci, 16 *Pseudomonas aeroginosa*, seven *Proteus mirabilis*, nine *Malassezia*

pachydermatis, and two *Candida* spp. were isolated from ear swab samples of dogs. In addition, they indicated that a 21.4% and 16.6% resistance rate was detected against chloramphenicol and gentamicin for all stains, respectively (Terziev & Urumova, 2018). Another study reported *Pasteurella* spp., *E. coli*, and *Proteus mirabilis* from 100 cats and 100 dogs' soral samples. Besides, the isolates were resistant to penicillin (11.53%) (Razali et al., 2020), in contrast to the current study result (33.3%).

Antimicrobial resistance is observed against almost all antibiotics in veterinary and human medicine (Hoang, et al., 2017). Recently, the increasing trend of antimicrobial resistance among bacteria due to their over and/or misuse of antibiotics for the treatment has been reported by investigators in previous studies (Srivastava et al., 2013; Nocera et al., 2021). Antimicrobial resistance is one of the most striking issues at the moment. Since antibiotic resistance spreads between bacteria, there will be an increase in bacterial-mediated diseases and clinical treatment failure, which is important for global public health (Adiguzel et al., 2021; Goulart et al., 2022; Baran et al., 2022). Similar to the previous report, moderately high antimicrobial resistance was detected in bacteria isolated in the current study.

Fungal agents were also reported in the previous study (Diren Sigirci et al., 2019). It has been reported that *Microsporum canis*, *Trichophyton* spp., *Microsporum gypseum, Trichophyton mentagrophytes, Microsporum nanum*, other *Microsporum* spp., and *Trichophyton tonsurans* were isolated from pet animals' skin lesion samples (Diren-Sigirci et al., 2019). However, *Aspergillus fumigatus*, *Penicillium* spp., and *Purpureocilium lilacium* were isolated from skin lesion samples collected from pet animals in the current study.

Conclusion

In summary, the current study emphasizes that the large, pet, and poultry animal samples were mostly submitted to the veterinary diagnostic laboratory between 2020 and 2021. An increasing trend of antimicrobial resistance was detected in the strains isolated from samples. These findings further emphasize that it is important to perform routine susceptibility testing in the veterinary diagnostic laboratory for the selection of appropriate antimicrobial therapy to prevent increasing antimicrobial resistance.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

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Veterinary Journal of Kastamonu University Case Report

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Cutaneous and ocular signs in a calf infected with *Theileria annulata* - case report

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Abstract: Tropical theileriosis is an important tick-borne disease in cattle that is caused by an obligate intracellular protozoan, *Theileria annulata* (*T. annulata*). In addition to the common clinical signs of tropical theileriosis such as pyrexia, lymph node enlargement, conjunctival petechiae, and anemia, veterinary practitioners may encounter unexpected clinical signs such as skin nodules and bilateral exophthalmos due to conjunctival edema. The main purpose of this report is to present clinical manifestations and histopathological findings of extensive cutaneous nodules and conjunctival edema in a calf infected with *T. annulata.*
Keywords: Calf, conjunctival edema, exophthalmos, skin nodules, tropical theileriosis

Theileria annulata ile enfekte bir buzağida kutanöz ve oküler bulgular

Özet: Tropikal theileriosis, obligat intrasellüler bir protozoon olan *Theileria annulata* **(***T. annulata*)'nın neden olduğu sığırların kene kaynaklı önemli bir hastalığıdır. Ateş, lenf yumrularında büyüme, konjuktivada peteşi ve anemi gibi klasik bulgulara ilaveten, klinisyenler deride nodül oluşumu ve konjunktiva ödeminden kaynaklanan bilateral ekzoftalmus gibi nadir gözlemlenen klinik bulgularla karşılaşabilir. Bu raporda *T. annulata* ile enfekte bir buzağıda yaygın deri nodülleri ve konjuktiva ödeminin klinik görünümü ve deri lezyonlarından alınan örneklerin histopatolojik bulguların sunulması konu edilmiştir.
Anahtar Kelimeler: Buzağı, konjunktiva ödemi, ekzoftalmus, deri nodülleri, tropikal theileriosis

Introduction

Tropical theileriosis is an important tick-borne disease in cattle that is caused by an obligate intracellular protozoan, *Theileria annulata* (*T. annulata*) (Brown, 1990). It is transmitted to cattle by Hyalomma ticks and particularly affects nonimmunized cattle. The disease is responsible for significant economic losses in the livestock industry in tropical and subtropical regions of the world, including Turkey (Uilenberg, 1995).

The clinical presentation of tropical theileriosis may vary from subacute to chronic disease depending on the host susceptibility, the quantity of the inoculated sporozoites, and the damaging effect of the pathogen on lymphoid tissue (Gill et al., 1977; Altay & Aktas, 2004). The common clinical signs are pyrexia, enlargement of superficial lymph nodes, lacrimation, nasal discharge, conjunctival petechia, and anemia (Gill et al., 1977; Aulakh & Singla, 2006). In addition to these classic signs of tropical theileriosis, the conjunctival edema and formation of nodular lesions on the skin in tropical theileriosis have been reported in a limited number of cases (Muhammad et al., 1999; Branco et al., 2010; Oryan et al., 2013; Gharbi et al., 2017). To the best of our knowledge, there is no report about the detailed presentation of conjunctival edema and cutaneous nodular lesions in Tropical Theileriosis in Turkey.

The main purpose of this report is to present clinical manifestations and histopathological findings of extensive cutaneous nodules and conjunctival edema in a calf infected with *T. annulata.*

Case Description

A 1-month-old calf was presented to Firat University Veterinary Teaching Hospital. The history revealed that the calf had dyspnea, inappetence, and weakness for 5 days. Physical examination findings included increased lung sounds, tachypnea (120 breaths/minute), tachycardia (180 beats/minute), increased body temperature (40.8 °C), bilateral enlargement of the superficial cervical lymph nodes, petechia on the muzzle, bilateral exophthalmos, conjunctival edema and icterus (Figure 1B-C). In addition, extensive cutaneous hemorrhagic nodules ranging in diameter from 0.5 to 2.0 cm were palpated on the skin over the entire body surface (Figure 1A).

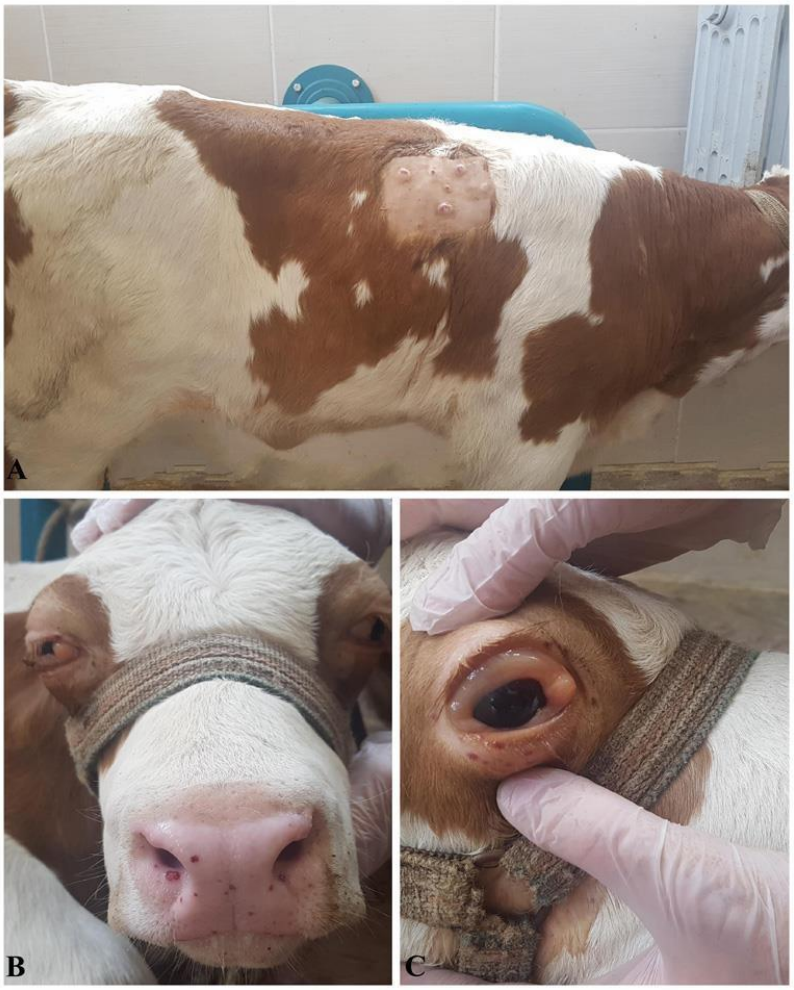

Figure 1: Cutaneous hemorrhagic nodules on the skin (A), Bilateral exophthalmos due to conjunctival edema and petechia on the muzzle (B), Conjunctival edema and icterus (C)

To examine the hematological changes, a 2 mL blood sample was collected from the jugular vein into the EDTA-containing tube and analyzed with an autoanalyzer (Prokan PE-6800 Vet, China). The peripheral blood smear was prepared from an ear tip blood sample and stained with Giemsa to examine the presence of blood parasites. In addition, two different skin nodules were excised for the histological examination. The nodule specimens were fixed in the formalin solution, embedded in paraffin, sectioned at 3 µm, stained with hematoxylin&eosin and examined using a light microscope (Olympus BX43, Tokyo, Japan). Histological examination showed hyperkeratosis, excessive thinning of the squamous epithelium layer, granuloma formations, lymphangitis, and severe lymphohistiocytic inflammatory cell infiltrations in the epidermis, dermis, subcutaneous muscle layer, and around hair follicles (Figure 2A-F).

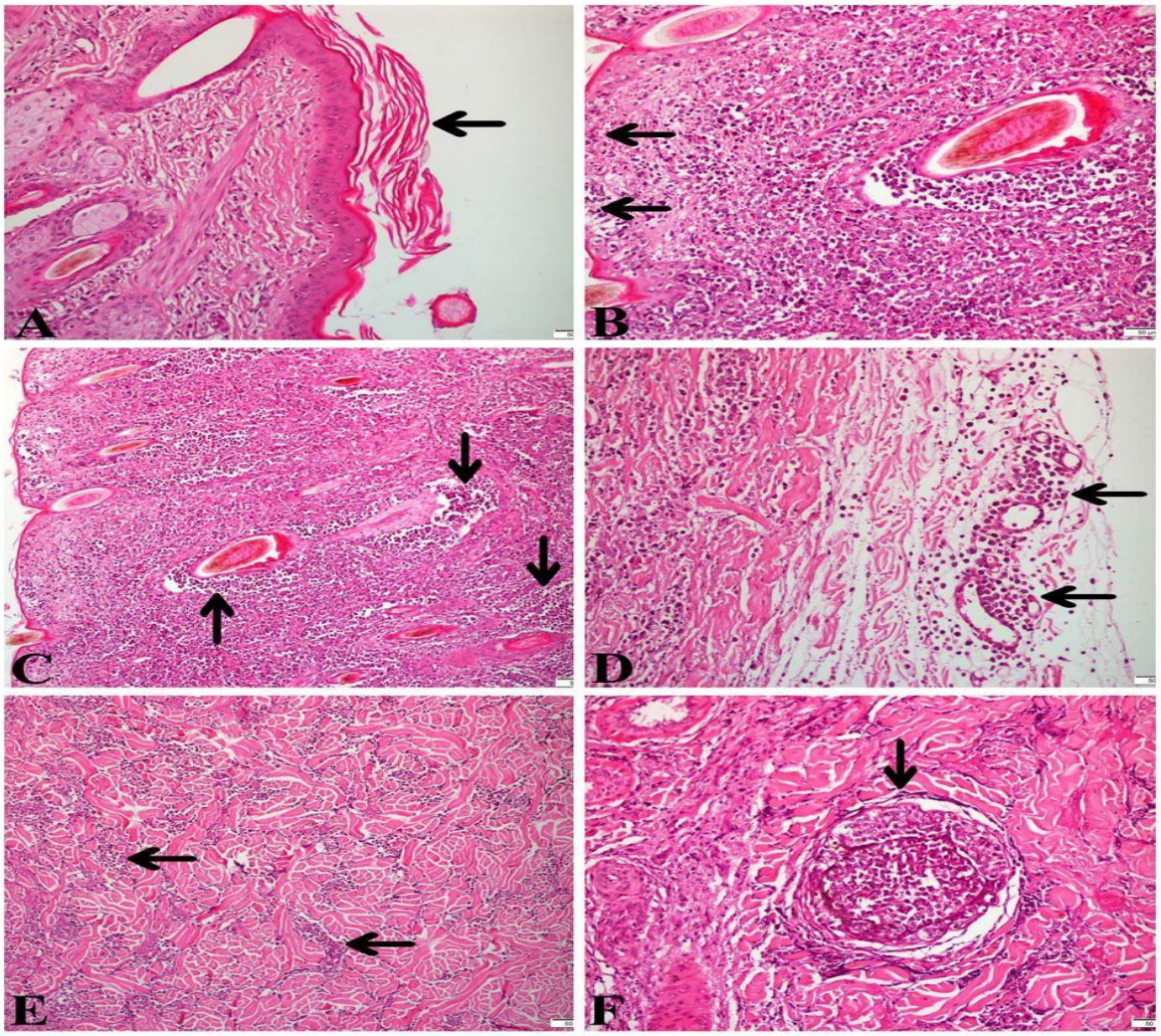

Figure 2: Hyperkeratosis (A), thinned epithelium layer (arrows) (B), severe inflammation in epidermis and dermis, particularly around the hair follicles (arrows) (C), lymphangitis, with mononuclear cells (arrows) (D), lymphohistiocytic inflammatory cells between the muscle fibers (arrows) (E) and a granuloma formation in muscle tissue (arrow) (F).

Hematological examination results revealed moderate anemia (Table). The peripheral blood smear was examined using a light microscope at 100x magnification and *T. annulata* piroplasms were detected within erythrocytes (Figure 3).

Table: The results of the hematological analysis

Parameters	Results	Reference*
WBC count (x 10^3/µL)	6.7	4-12
RBC count (x 10^6/µL)	4.45	5-10
Hemoglobin (g/dL)	6.2	8-15
Hematocrit (%)	20	24-46
MCV (fL)	52.18	40-60
MCHC (g/dL)	26.8	30-36
Thrombocytes (x 10^9/µL)	309	100-800

WBC: white blood cell; RBC: red blood cell; MCV: mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration; *(Radostits et al., 2007)

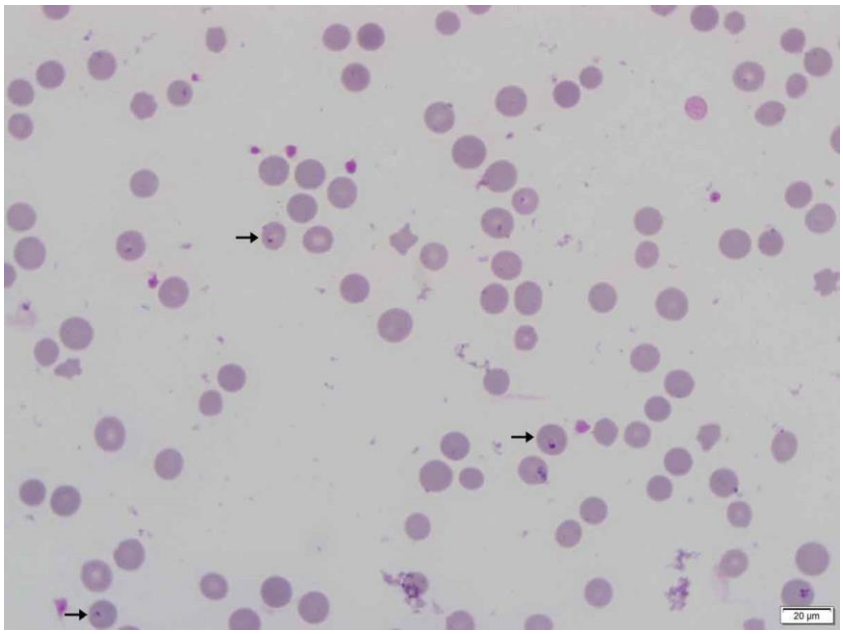

Figure 3: *Theileria annulata* piroplasms within erythrocytes (arrows)

Based on the clinical findings and the results of the hematology and blood smear, the calf was tentatively diagnosed with Tropical Theileriosis. To confirm the presence of *T. annulata*, nested PCR was performed. The primers Nbab1F (5′-AAGCCATGCATGTCTAAGTATAAGCTTTT-3′) and Nbab1R (5′-CCTCTCCTTCCTTTAAGTGATAAGGTTCAC-3′) were used for first amplification of an approximately 1600 bp fragment of the 18S rRNA gene in Theileria and Babesia spp. (Oosthuizen et al., 2009). The nested amplification performed using the reverse line blotting

(RLB) primers RLB-F2 (5′-GACACAGGGAGGTAGTGACAAG-3′) and RLB-R2 (5′-biotin CTAAGAATTTCACCTCTGACAGT-3′) generated 492–498 bp-long fragment from the V4 hypervariable region of the 18S ribosomal RNA (rRNA) gene (Georges et al., 2001). According to the RLB assay, the sample was found to be infected with *T. annulata*.

The calf was treated with a single injection of buparvaquone (Butalex, MSD Animal Health, Turkey) at a dose of 2.5 mg/kg and a daily injection of oxytetracycline (Primamycin LA, Zoetis, USA) at a dose of 20 mg/kg. The calf gradually recovered, and cutaneous nodules and conjunctival edema completely disappeared after 25 days of the treatment.

Discussion

Conjunctival edema and the formation of nodular lesions on the skin are unusual clinical findings in Tropical Theileriosis. A previous study performed on 112 cattle with Tropical Theileriosis revealed that only 9.8% and 14.3% of the clinical cases showed nodular lesions on the skin and conjunctival edema, respectively (Muhammad et al., 1999). Whereas, in the same study, the distribution of classical signs such as pyrexia, enlargement of superficial lymph nodes, lacrimation, and anemia were found 100%, 88.4%, 71.4%, and 42.7%, respectively. Because of the low frequency of dermatological and ocular findings, veterinary practitioners may overlook these clinical signs unless they are seen in conjunction with classic findings such as fever, lacrimation, enlargement of superficial lymph nodes, and anemia.

The results of the histopathological examination of nodule specimens revealed hyperkeratosis, excessive thinning of the squamous epithelium layer, granuloma formations, lymphangitis, and severe lymphohistiocytic inflammatory cell infiltrations in the epidermis, dermis, subcutaneous muscle layer and around the hair follicles. In a previous report, the researchers stated that pathological lesions that occurred in different organs and tissues are caused by the proliferation of the infected macrophages (Forsyth et al., 1999). In another study, uninfected mature T lymphoid cells and macrophages were detected in different organs (Branco et al., 2010). In that study, the authors also noted that pathological lesions may be associated with the overproduction of interferon gamma (IFN-γ) and (interleukin) IL-2 by naïve T cells, leading to the proliferation of infected and noninfected macrophages. Some researchers showed the presence of intra- and extracellular schizonts with immunohistochemistry of the nodules by labeling tissue sections with an antibody (Mab 1C7) that reacts with schizonts in the tissue sections (Oryan et al., 2013; Branco et al., 2010).

Conclusion

Veterinary practitioners should consider Tropical theileriosis in the differential diagnosis when they encounter the signs such as skin nodules and conjunctival edema.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

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Clinical, radiographic, ultrasonographic diagnosis, and treatment of urolithiasis in two domestic cats - case report

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Abstract: In this case report, clinical, radiographic, and ultrasonographic diagnosis, and operative treatment of uroliths encountered in the urinary bladder of two domestic cats were shared. In the radiographic and ultrasonographic examinations of both cats brought with complaints of intermittent urination, uroliths were detected in their urinary bladders. After the uroliths were removed by cystotomy operation, it was determined that both cats were followed for six months and were in good health and no recurrence was observed.
Keywords: Cat, cystotomy, ultrasonography, urolithiasis

Evcil iki kedide karşılaşılan ürolitlerin klinik, radyografik, ultrasonografik tanısı ve tedavisi

Özet: Bu olgu sunumunda, iki evcil kedinin idrar kesesinde karşılaşılan ürolitlerin klinik, radyografik ve ultrasonografik tanısı ile operatif tedavisi paylaşıldı. Kesik kesik idrar yapma şikâyetleri ile getirilen her iki kedinin radyografik ve ultrasonografik muayenelerinde idrar keselerinde ürolitler tespit edildi. Sistotomi operasyonu ile ürolitler uzaklaştırıldıktan sonra altı ay boyunca takip edilen her iki kedinin de sağlık durumlarının iyi olduğu ve nüks gözlenmediği belirlendi.
Anahtar Kelimeler: Kedi, sistotomi, ultrasonografi, ürolithiazis

Introduction

Urolithiasis is one of the most common lower urinary system problems in cats and dogs. Uroliths are formed as a result of the accumulation in the urinary system by reaching supersaturation of the minerals that need to be removed from the body with urine. It is named nephrolith, renolite, ureterolite, urecystolite, and urethrolite, depending on where it occurs. According to the mineral composition it contains, uroliths are named calcium oxalate, magnesium ammonium phosphate, cystine, and urate (Koehler et al., 2008; Sancak et al., 2009; Albasan et al., 2013; Tiruneh & Abdisa, 2017; Kopecny et al., 2021).

Many pathological and physiological factors affect the formation of uroliths. Anatomical and metabolic abnormalities, poor diets, urinary tract infections, and changes in urine pH are

important factors in the formation of uroliths. Some demographic factors such as species, race, age, gender, and sterilization may predispose to urolith formation. For example; it has been reported that urinary crystal is detected more frequently in dogs, mostly in breeds such as Miniature schnauzer, Shih tzu, Lhasa apso, and Yorkshire terrier. In cats, urinary crystals are more likely to be seen in domestic shorthairs (such as British shorthairs), Himalayan, Persian, and Ragdoll breeds. According to the Minnesota Urolith Center, the most common uroliths in dogs is struvite, while in cats calcium oxalate crystals (Lulich et al., 2000; Osborne et al., 2000; Houston & Moore, 2009; Albasan et al., 2013).

Urolithiasis causes clinical symptoms such as intermittent and painful urination, hematuria, and frequent urination position in cats and dogs. Sometimes, complaints such as the inability to urinate occur due to obstruction of the urethra. In these cases, kidney dysfunction occurs due to the inability to urinate, resulting in hydronephrosis and uremia. In advanced stages, it can cause irreversible fatal complications such as urinary bladder rupture (Sancak et al., 2009; Tiruneh & Abdisa, 2017; Küçük, 2020).

In the diagnosis of urolithiasis, besides anamnesis and clinical findings, the use of ultrasonography together with direct and indirect radiography is important. The fact that the urinary bladder creates a natural contrast due to its physiological structure enables the diagnosis to be made easily in direct radiography applications. In the ultrasonographic examination, it is detected in the urinary bladder as masses that have a hyperechoic appearance and cause acoustic shadow artifacts (Rinkardt & Houston, 2004; Langston et al., 2008; Sancak et al., 2009; Albasan et al., 2013; Tion et al., 2015; Tiruneh & Abdisa, 2017).

Cystotomy is the most commonly used operation in the treatment of uroliths (urecystolitis) in the urinary bladder. In urolithiasis cases, the patient's blood values are very important in the preoperative period. In particular, the presence of uremia and hyperkalemia, metabolic acidosis, and serum creatinine levels should be carefully monitored. In the postoperative period after cystotomy, antibiotic applications for urinary tract infections and urinary tract antiseptics should be applied. Again in this period, if the urine pH value is alkaline, it will be beneficial to acidify the urine by applying ascorbic acid. Otherwise, this situation should be carefully evaluated, as ascorbic acid will cause the recurrence of urinary stones (Sancak et al., 2009; Albasan et al., 2013; Tion et al., 2015).

This study, it was aimed to convey our experience to our colleagues and contribute to the literature by discussing the diagnosis and treatment stages of urolithiasis cases detected in two domestic cats.

Case Description

In this case report, the diagnosis and operative treatment of uroliths encountered in the urinary bladders of two domestic cats brought with the complaint of intermittent urination were discussed. The information obtained in line with both cats' anamnesis and laboratory examination is presented in Table.

Table: Findings obtained in accordance with the anamnesis and laboratory examination of the patients

	Case 1	Case 2
Breed	Crossbreed	Crossbreed
Gender	Female	Male
Age	6 years	2 years
Sterilization	+	-
Diet	Urinary cat food (containing 34% protein)	Home cooking
Complaint	Intermittent urination for 1 week	Difficulty urinating
Disease history	2 months ago urinary tract infection	Healthy
Water consumption	About 300 ml of purified water	Tap water
pH of urine	6.0	5.0

In clinical examination with palpation, it was determined that the urinary bladders of both cats were full and they urinated with a massage. In the radiographic examination, solid foreign masses with a radiopaque appearance were detected in the urinary bladder of the cats (Figure 1-A, B).

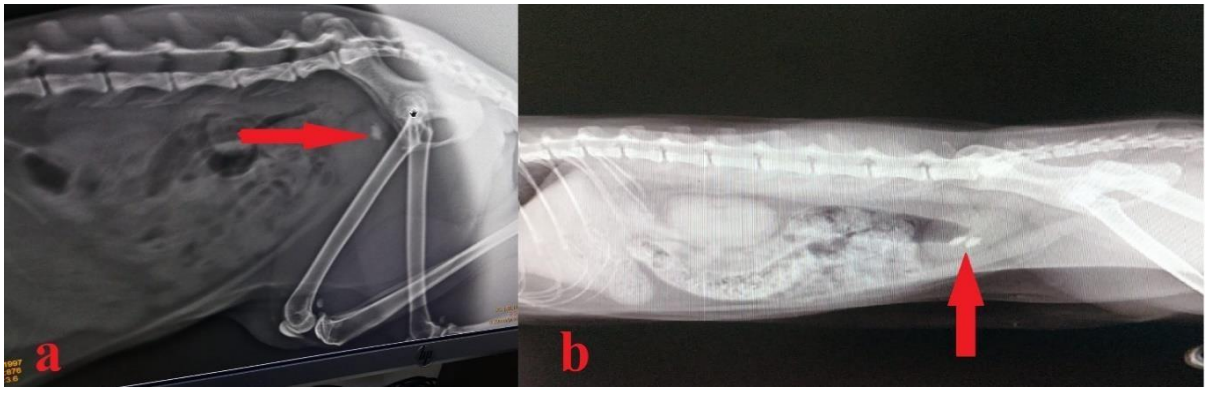

Figure 1: Uroliths (arrows) were detected on latero-lateral abdominal direct radiographs of cats with urolithiasis; case 1 (A), case 2 (B).

In the ultrasonographic examination, hyperechoic masses forming acoustic shadow artifacts were detected in the urinary bladders of both cats (Figure 2-A, B). According to the findings obtained as a result of anamnesis, clinical examination, and imaging methods, both cases were diagnosed with urolithiasis. While a single urolith was detected in case 1, two uroliths were detected in case 2.

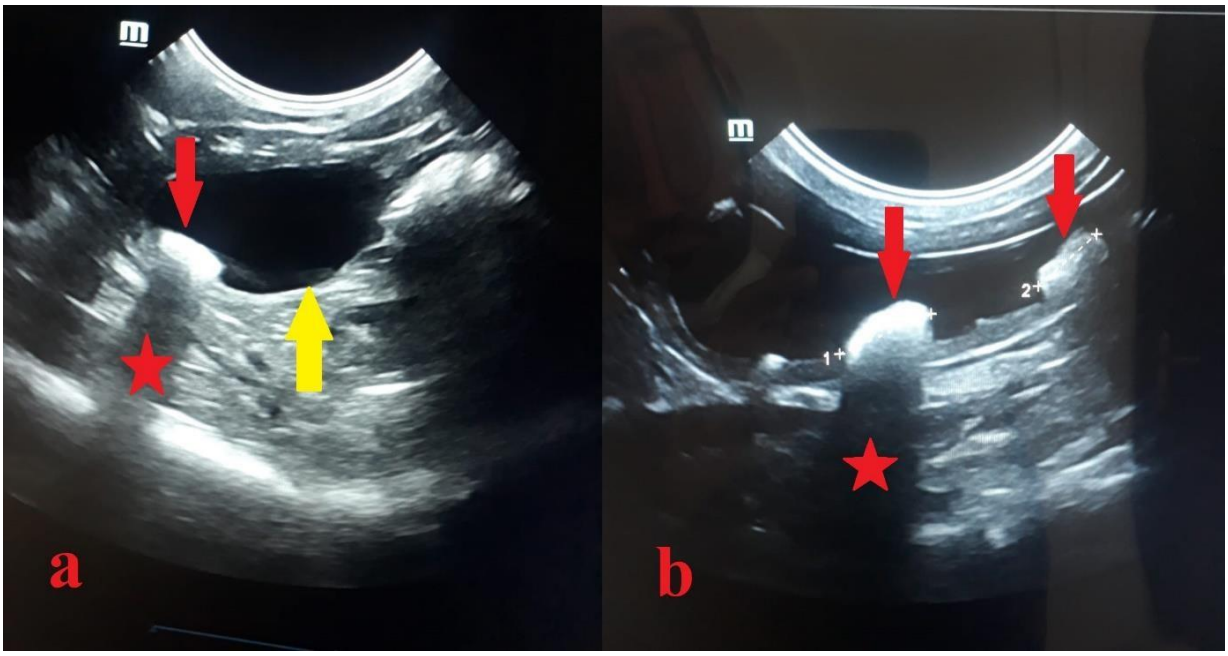

Figure 2: Uroliths (red arrows) and acoustic shadow artifacts (stars) were detected on abdominal ultrasonography of cats with urolithiasis; case 1 (A) and case 2 (B), urinary bladder (yellow arrow).

It was decided to remove the uroliths by performing a cystotomy operation on both cats with the diagnosis of urolithiasis. Before the operation, the patients were evaluated in terms of hyperkalemia, acidosis, and uremia. Since no abnormality was detected in the blood values of both patients, it was deemed appropriate to be operated on immediately. For the cystotomy operation, the operation area was prepared by shaving the abdomen from the level of the processus xiphoideus to the pubis. The patient was then placed on the operating table in the ventrodorsal position. A 2-3 cm long skin incision was made on the median line from the caudal of the umbilical cicatrix to both cats. Fat and connective tissue were bluntly dissected until the linea alba was visible. After exposing the linea alba, the abdomen was opened with scissors with a blunt end. Then, the urinary bladder was taken out from the abdomen with slow movements. The urinary bladder, which was surrounded by absorbent sponges, was opened by making a 0.5 cm long incision on its long axis from the region with the least vascularization. After removing the stones in the urinary bladder and washing the bladder with the help of

anatomical forceps, a 1.0 mm catheter was placed from the urinary bladder to the urethra by the anterograde route and it was checked whether there was an obstruction in the urethra. After cleaning the uroliths in the urinary bladder and urethra, the urinary bladder was closed with absorbable suture material (Vicryl, USP 3-0, USA) using *Schmieden* and *Cushing* sutures. After making sure that there was no leakage from the bladder, the abdomen was closed with a standard surgical procedure. Postoperatively, amoxicillin-clavulanic acid (Synulox, Zoetis, Turkey) was administered intramuscularly at a dose of 8.75 mg/kg/day. In addition, methenamine (Purinol, Recordati İlaç San., Turkey) at a dose of 50 mg/kg was administered orally, divided into three, during the day. In the controls performed on the 15^{th}, 30^{th}, 60^{th}, and 180^{th} days, it was determined that the general condition of the cats was good and there were no signs of recurrence of uroliths.

Discussion

Urolithiasis is a chronic lower urinary system problem in many animal species, which is characterized by intermittent urination, pain during urination, and hematuria (Küçük, 2020; Aké-Chiñas et al., 2022). It has been reported that urolithiasis is detected in 25% of cats with lower urinary system problems (Sancak et al., 2009). In this case report, it was learned that both cats had complaints of intermittent urination.

Studies are reporting that the incidence of urolithiasis cases in males and females is equal (Grauer, 1993). However, in some studies (Houston & Moore, 2009), it has been reported that urolithiasis cases are more likely to be seen in females, and some studies (Sancak et al., 2009) in males. In this study, one of the two cats with urolithiasis is male and the other is female.

Some types of uroliths are formed mostly in acidic urine (such as urate, uric acid, and calcium oxalate), while some types of uroliths are formed mostly in alkaline urine (such as struvite, calcium carbonate). Urine pH in cats and dogs varies between 6.0-7.5 (Rizzi, 2014). In one study, the urinary pH of cats was reported as 6.0 - 6.4 (Küçük, 2020). In this case report, one of the cats had a urinary pH of 6.0, while the other had a urinary pH of 5.0. The diets used in the nutrition of cats are very important in the formation of urolithiasis. In this study, it was determined that one of the cats with urolith was fed with a commercial food containing 34% crude protein developed against urinary diseases, and the other was fed with homemade food.

In the diagnosis of urolithiasis cases, direct and indirect radiography and ultrasonography are very important. The formation of a natural contrast due to the contents of the urinary bladder allows easy detection of radiopaque uroliths on direct radiographs. Again, in ultrasonographic examinations, uroliths in the urinary bladder can be detected as masses with a hyperechoic

appearance and an acoustic shadow artifact (Rinkardt & Houston, 2004; Langston et al., 2008; Sancak et al., 2009; Albasan et al., 2013; Tion et al., 2015; Tiruneh & Abdisa, 2017). In this case report, uroliths in the urinary bladder of both cats were revealed by direct radiography and ultrasonography.

Urolithiasis cases in the urinary bladder can be treated conservatively and operatively. In conservative treatment, appropriate diet applications are made according to the type of urolith. At the same time, urinary tract antiseptics and parenteral antibiotics are also applied. Again, ascorbic acid applications can be made in some urolith types (such as struvite), and allopurinol (xanthine oxidase inhibitor) applications can be made in some urolith types (such as urate). Uroliths, which are difficult to dissolve in the urinary bladder, obstruct the passage to the urethra and make urination difficult, may need to be removed with a cystotomy operation (Sancak et al., 2009; Albasan et al., 2013; Tion et al., 2015; Küçük, 2020). In this case report, it was decided to perform a cystotomy because conservative treatment of uroliths detected in both cats was not possible. As a result of the cystotomy, a single urolith was removed in one of the cats and two in the other.

Conclusion

It has been revealed that the importance of direct radiography and ultrasonography in the diagnosis of urinary bladder uroliths, the importance of diets applied to cats in urinary stones and cystotomy is a viable treatment options.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

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Negative effects of zearalenone on reproductive productivity in dairy cattle

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Abstract: Mycotoxins, which are produced by different fungi reproduced in feed materials worldwide, have adverse effects on animal and human health. Zearalenone (ZEN) is a common mycotoxin, secreted from *Fusarium* spp. that may cause reproductive problems in animal species due to its non-steroidal estrogenic feature. The sensitivity of livestock to ZEN differs based on the species and sexuality. Cows are considered less sensitive to ZEN, however, there is limited information about adverse effects caused by ZEN. In this article, we aimed to review the effects of ZEN on cows in negative energy balance.
Keywords: Zearalenone, α-zearalenol β-zearalenol, negative energy balance, dairy cattle

Zearalenone'nun süt sığırlarındaki üreme verimliliği üzerine olumsuz etkileri

Özet: Dünya genelinde yem materyellerinde yaygın olarak üreme imkanı bulan farklı mantar türleri tarafından üretilen mikotoksinlerin, hayvan ve insan sağlığı üzerinde olmusuz etkileri bulunmaktadır. Zearalenone (ZEN), *Fusarium* türleri tarafından üretilen ve hayvan türlerinde üreme problemlerine neden olabilen non-steroidal östrojen özelliğine sahip yaygın bir mikotoksindir. Çiftlik hayvanlarında bu mikotoksine karşı duyarlılık tür ve cinsiyet bazında farklılık göstermektedir. Süt inekleri, ZEN' e karşı en az hassas hayvanlar olarak düşünülmektedir. Bununla birlikte ZEN tarafından oluşturulan olumsuz etkiler üzerine sınırlı sayıda veriler bulunmaktadır. Bu derlemede, yüksek verimli süt ineklerinde görülen negatif enerji dengesinden ileri gelebilecek üreme kayıplarına, ZEN'un ilave etkisi araştırılmaya odaklanıldı.
Anahtar Kelimeler: Zearalenon, α-zearalenol β-zearalenol, negatif enerji dengesi, süt sığırı

Introduction

Zearalenone (ZEN) is one of the most common mycotoxins that is produced by *Fusarium* spp. mainly *F. graminearum*, *F. culmorum*, and *F. vericillioides* (Ropejko & Twaruzek, 2021). ZEN is a non-steroidal estrogen mycotoxin that contains resorcylic acid lactone and ketone groups in structure and has a close structural relationship with other antibiotic metabolites that are produced by a number of microscopic filamentous fungi (Desjardins & Proctor, 2007). ZEN is a crystalline toxin with a melting point of 159-163 °C (Harcarova et al., 2020).

Approximately 25-50% of crops are contaminated with different mycotoxins exceeding the EU (European Union) and Codex limits worldwide. Actually, this figure greatly undervalues the occurrence above the detectable levels (Ricciardi et al. 2013; Eskola et al., 2020). Suitable conditions for ZEN production by fungi are when humidity is above 20% and temperatures ranging from 20 to 25 °C within a three weeks period. The countries with warm and wet climates are favorable for high levels of ZEN generation in feedstuffs (Mostrom, 2012). A survey study on ZEN contamination of cereals between 1999 and 2008 showed that 83.3% of samples in China at concentrations ranging from 46 to 3079 μg/kg (Li et al., 2021). ZEN and its metabolites are heat stable, and thus thermal breakdown in structure is unlikely to occur during the manufacturing and pelleting of compound feeds. ZEN is stable up to 120 °C, and heating temperatures at 150 °C and 200 °C for 60-min a degradation in ZEN structure occurred 29% and 69%, respectively (Kuiper-Goodman et al., 1987).

Zearalenone has a competition to bind to estrogen receptors due to structural similarities to that of naturally occurring estrogens such as estradiol, estrone, estriol, and 17β-estradiol and exerts estrogenic effects in different animal species (Figure 1) (Metzler et al., 2010; Gromadzka et al., 2008; Edite Bezerra da Rocha et al., 2014; Martins et al., 2020). Additionally, animal species have different sensitivity, including the gender differences, to zearalenone with female pigs being more sensitive than male pigs, followed by sheep, cattle, and poultry (EFSA, 2017). Generally, mycotoxins exert their toxic effects less individually, and the co-occurrence nature of mycotoxins in a feedstuff during harvesting or storage conditions may enhance their toxicity. In a comparison study on the toxic effects of ochratoxin and ZEN although the receptors for these mycotoxins are different, it has been reported that these toxins have mechanistic overlap via the generation of reactive oxygen species, which leads to amplifying cellular toxicity (Li et al., 2014). Antagonistic effects may also occur between toxins. Alassane-Kpembi et al. (2015) observed that deoxynivalenol (DON) and fusarenon-X have an antagonistic impact while nivalenol and fusarenon-X have an agonism. In addition, these agonistic/antagonistic effects between different mycotoxins exist depending on their concentrations. Zheng et al. (2018) reported that ZEN and α-zearalenol was synergistic at high concentrations, whereas they were antagonistic at low concentrations.

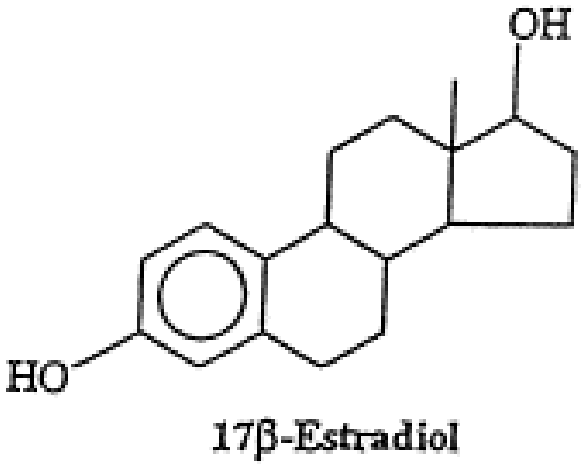

Figure 1: Chemical structure of 17β-estradiol.

Degradation of ZEN in the rumen and other tissues

Ruminants may be exposed to different mycotoxins due to their complex ration contents that originate from different feed materials that include roughage and grains. Unlike monogastric animals, rumen microbiota can degrade some mycotoxins to less toxic or non-toxic metabolites by enzymes that are released by the microorganisms. In the detoxification process, the oxygen in the epoxide group of DON is removed to give a carbon-carbon double bound resulting in non-toxic de-epoxy metabolites (King et al., 1984). The newly formed metabolite is called de-epoxy DON, and the toxicity test showed that de-epoxy DON was 500 times less toxic than the parent metabolite (Schatzmayr et al., 2006). In contrast to DON detoxification and metabolism of ZEN in the rumen fluid by conjugation with glucuronic acid can be converted to toxic metabolites α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL), which are 60 times and 0.2 times more toxic, respectively (Figure 2) (Kiessling et al., 1984; Seeling et al., 2006; EFSA, 2016; Mirocha et al., 1978b). α-ZEL exerts a higher estrogenic effect in comparison to the parent compound. Thus, the conversion of ZEN to β-ZEL may be a deactivation process, whereas the conversion to α-ZEL may be an activation process of parent metabolites (Figure 3). This conversion of ZEN to α-ZEL and β-ZEL ratios was observed between 2:1 and 3:1 after an incubation period of 24 h (Valenta & Vemmer, 1996). Danicke et al., (2005) reported that 89% of administrated 0.1 mg ZEN/kg diet was recovered at the proximal duodenum as 30% ZEN, 30% α-ZEL, and 40% β-ZEL. Moreover, α-ZEL and β-ZEL are also produced by *Fusarium* spp. in much lower concentrations in comparison to ZEN production (Zhang et al., 2018). Following absorption from the digestive tract, ZEN is converted to its metabolites in different tissues such as the liver and ovary. In a study conducted

by Malekinejad et al. (2006), it has been demonstrated that ZEN is converted to α-ZEL and β-ZEL in porcine and bovine granulosa cells, respectively. In another word, the preferential biotransformation of ZEN to either α-ZEL or β-ZEL in the liver and different tissues may suggest species differences in the sensitivity to the estrogenic effect of ZEN and its metabolites for animal species.

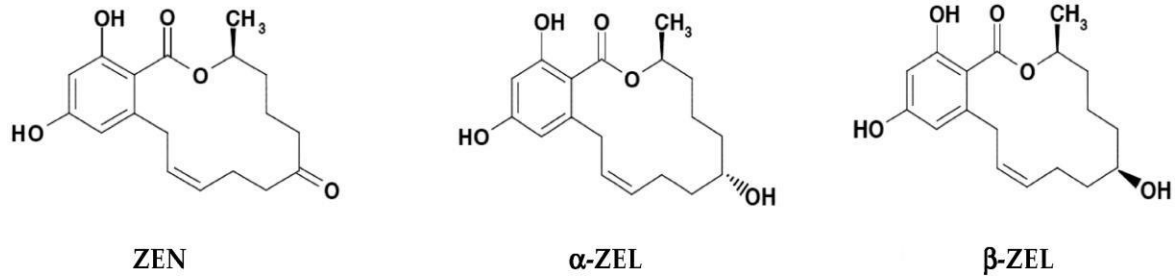

Figure 2: The chemical structure of zearalenone, α-zearalenol, and β-zearalenol (Ropejko and Twaruzek, 2021)

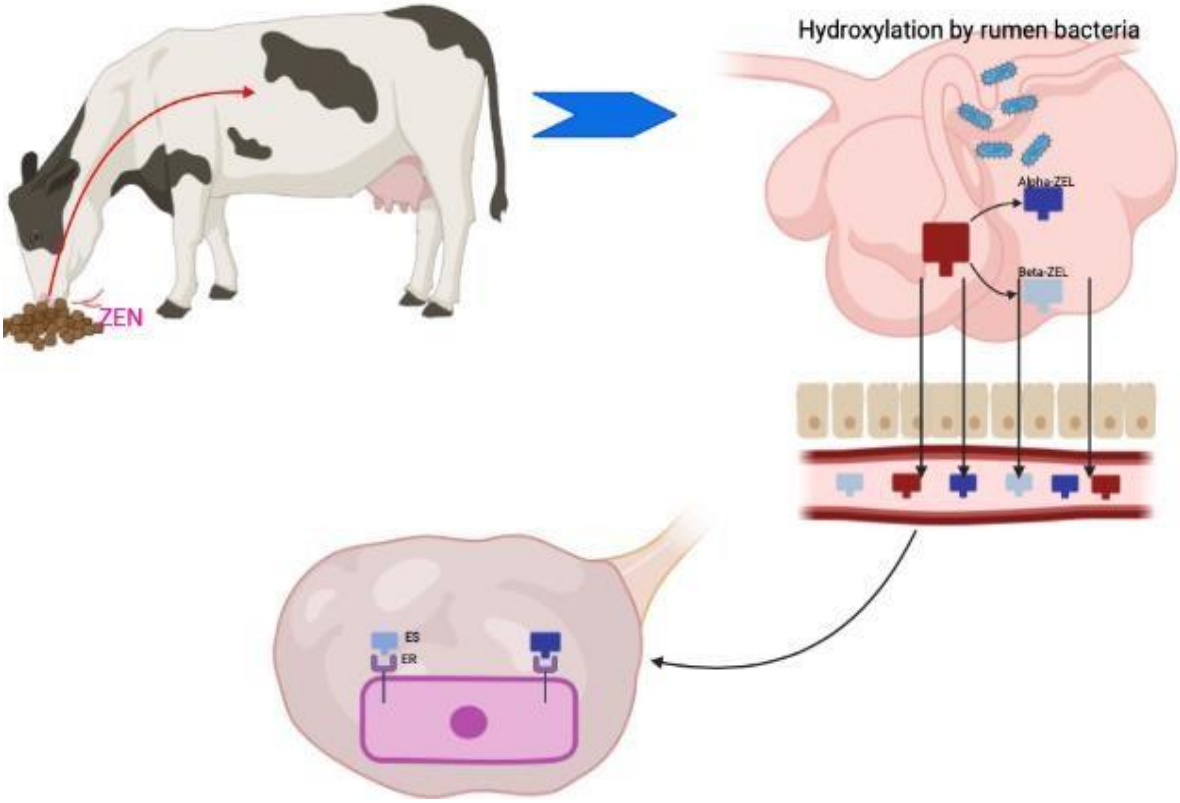

Figure 3: An illustration of ZEN metabolism in the rumen. ZEN and *its* metabolites bind to estrogen receptors and exert estrogenic effects on animals. 17 beta-estradiol and ZEN-and *its* metabolites bind to estrogen receptors

Negative energy balance in dairy cows

Up to date, high-yielding dairy cows are becoming more susceptible to negative energy balance (NEB) and are accompanied by metabolic disorders arising from the significant increase in milk production per lactation as a consequence of genetic selection and improved nutritional management. For dairy cows, the most critical period is the transition period since the energy demand for maintenance and lactation exceeds that provided with dietary energy intake (Bauman & Currie, 1980). During this period, dry matter intake (DMI) is reduced between 20-35% a few days prior to calving and remains low till the lactation peaks (Grummer et al., 2004; Hayirli et al., 2002; Marquardt et al., 1977). During this period body condition score loss occurs that is accompanied by excessive body tissue mobilization to compensate for the energy requirement. Regaining the body mass and the metabolic profile to the normal levels may take up to 20 weeks after lactation onset (Taylor et al., 2003). During the transition period, dairy cows with NEB are vulnerable to perform reproductions problems such as retained fetal membranes, clinical and subclinical metritis, and decreased conception rates (Huzzey et al., 2015; Castro et al., 2012; Moretti et al., 2016; Kumari et al., 2016; Civelek et al., 2011; Shin et al., 2015). In addition to low circulating Insulin-like Growth Factor (IGF)-I after calving, NEB may also influence IGF availability in the oviduct indirectly through changes in specific IGFBP (IGF-Binding Protein) expression. It is possible that the predicted increased signaling by IGF-II may perturb embryo development, contributing to the high rates of embryonic mortality in dairy cows (Fenwick et al., 2008). Plasma insulin concentrations and insulin sensitivity can decrease during the periparturient period, followed by an increase in plasma non-esterified fatty acid concentrations (Wankhade et al., 2017). Kinoshita et al. (2018) have reported that long-term DON consumption (5 mg/kg dry matter) induced mild changes in energy metabolism in lactating dairy cows. For avoiding the detrimental effects of NEB in dairy cows, some strategies have been developed such as enrichment of rations with some ingredients (Overton and Waldron, 2004). Feeding management that is applied to alleviate NEB contains a high level of concentrate feed. In addition, ZEN and other common mycotoxins are produced in numerous substrates including wheat, barley, corn, rice, sorghum, and corn silage more than roughages, and at the same time, concentrated feedstuffs harbor the highest level of mycotoxin (Tola & Kebede, 2016). Thus, consuming a high level of concentrated feedstuffs by transition dairy cows may expose these animals to high levels of mycotoxins including ZEN.

Some substances, which are responsible for the specific moldy odor, may be produced by particular fungi species, and are called microbial volatile organic compounds. Up to date, about 150 volatile compounds have been described (Fiedler et al., 2001). Feeding times are prolonged,

and feed intake is reduced in cattle since cattle also dislike the moldy odor when these compounds are present in feedstuffs and total mixed ration (Fink-Gremmels, 2008). During the periparturient period, this moldy scent may further contribute to the NEB in dairy cattle.

Reproduction in dairy cows

Due to its lipophilicity, which is reflected by a relatively high log partition coefficient between octanol and water (log K_{ow}), ZEN is rapidly absorbed in the small intestines following oral exposure. The apparent volume of distribution is large and includes target tissues of ZEN toxic action, such as the uterus, ovarian follicles, and testes (Kuiper-Goodman et al., 1987). Animals eliminate the ZEN via urine, and feces exclusively. In contrast to aflatoxin M1 and DON, ZEN carry-over on milk is not related to the milk yielding (Danicke & Winkler, 2015). As previously mentioned, ZEN has structural similarities to that of naturally occurring estrogens and competition among them, therefore, ZEN plays important role in reproductive disorders in domestic animals, particularly in pigs (Metzler et al., 2010; Gromodzka et al., 2008; EFSA, 2017). Although there are limited publications on cattle sensitivity to ZEN, these experiments have determined infertility, vaginitis, enlarged vulvae, vulvo-vaginitis, reduced milk production, and hyperestrogenism (Coppock et al., 1990; Minervini et al., 2001). Although pigs are considered the most sensitive to ZEN, similar symptoms may be observed in calves that have undeveloped rumen functions, and young heifers (Kallela & Ettala, 1984). In contrast, Silva et al. (2021) have observed no change in morphometric parameters of the genital tract of the beef heifer that consumed a diet contaminated with 300 ppb (~8.82 μM).

Mirocha et al. (1968) have reported that administration of hay containing 14 ppm ZEN caused infertility in cattle. Additionally, cattle and sheep that were grazing in contaminated pastures, have demonstrated infertility (Towers & Sprosen, 1993). In another study conducted by Roine et al. (1971), it has been verified that ZEN-administered cows were infertile. For 42 consecutive days, cows that were administered ZEN at concentrations of 25 or 100 ppm exhibited swollen and hyperemic external genitalia but estrous cycles and ovulations were normal in the trial (Mirocha et al., 1978a). Due to the estrogenic effect, mammary gland enlargement and gland development in heifers occurred in the herds that consume ZEN-contaminated feedstuffs (Coppock et al., 1990; Bloomquist et al., 1983). The artificial insemination index was increased when contaminated feeds were consumed by dairy herds (Kramer, 1997). Moreover, Weaver et al. (1986) have observed a decrease in conception rates in dairy heifers fed 250 mg of ZEN for three estrous cycles.

Following the ingestion of ZEN, parent compounds and their metabolites can be detected in follicular fluids in the ovary in different concentrations (Winkler et al., 2015; Takagi et al., 2008; Malekinejad et al., 2006). In the ovary, ZEN may exert its detrimental effects in different ways in cattle. In an *in vitro* study, it was observed that α-ZEL or ZEN (94μM) inhibited the maturation of oocytes to metaphase II with a significant increase in chromatin abnormalities (Minervini et al., 2001). In contrast, Takagi et al. (2008) have not observed a difference in the occurrence of metaphase II in oocytes that were exposed to ZEN with <0.31 μM. Thus, dose-dependent exposure is important for the maturation rates in cattle oocytes. However, it has been observed that the maturation rates in the oocytes that were exposed to ZEN with 3.1 μM are significantly decreased during the trials. Beef heifers fed a diet contaminated with 300 ppb ZEN had significantly reduced viable oocytes compared with the control group (Silva et al., 2021). Anti-Müllerian hormone is a glycoprotein produced by the granulosa cells and inhibits the primordial follicles to turn into the growing follicles, in addition, decreases the responsiveness of follicles to follicle-stimulating hormone (FSH). Anti-Müllerian hormone is considered to be a marker of the population of small antral gonadotropin-responsive follicles in cows (Monniaux et al., 20013; Visser and Themmen, 2014; Monniaux et al., 2014; Rico et al., 2009). A study carried out by Fushimi et al. (2015) observed that two different herds with dietary ZEN contamination below the admissible levels showed a significant difference in anti-Müllerian hormone which indicate differences in the antral follicle populations between herds. Thus, ZEN, even at low levels, may alter the ovarian antral follicle populations, but not fertility after the artificial insemination, of post-partum cows.

Zhu et al. (2012) observed apoptosis and necrosis of granulosa cells via a caspase-3 and caspase-9-dependent mitochondrial pathway in a mouse. It suggests that ZEN and its metabolites also may induce atresia in ovarian follicles of dairy cattle. A study carried out by Yang et al. (2019) corroborates the apoptotic effect of ZEN in bovine ovarian granulosa cells. In this study, β-ZEL and HT-2 inhibited cell proliferation in a dose-dependent manner and induced apoptosis in bovine granulosa cells. The individual effect of β-ZEL or α-ZEL on apoptosis in the bovine ovarian granulosa cells requires further investigation.

G protein-coupled estrogen receptor (GPR30) present in cattle gonadotropes is responsible for rapid negative estradiol feedback regulation of GnRH-induced luteinizing hormone (LH) secretion in cattle (Rudolf and Kadokawa, 2013). Nakamura et al., (2015) observed that α-ZEL may suppress LH secretion from the anterior pituitary of cattle via GPR30. In beef heifers offered ZEN-contaminated ration, plasma estrogen concentrations were not

affected (Silva et al., 2021). In addition, α-ZEL had no effect on cell proliferation in the presence of IGF-I and FSH, inhibited progesterone and estradiol, whereas decreased cell numbers in the presence of FSH alone, and had no effect on progesterone and estradiol production (Pizzo et al., 2016). In granulosa cell cultures that were exposed to α-ZEL for 24 hours, 17β-estradiol levels were found increased is related to the inhibitory effects of the ZEN pathway of steroidogenesis (Minervini et al., 2001).

Conclusion

Understanding mycotoxins features and their effects on animal health, production, and reproduction parameters become crucial for the feed industry and livestock management. High-yielding dairy cows are more susceptible to NEB, leading to decreased lactational and reproductive performance. The feeding management that is aimed at alleviating these adverse effects of NEB may be disrupted by the mycotoxin presence in feedstuffs. Mycotoxins may be augmenting the adverse effects of NEB via hormonal and functional changes in the hypothalamic-ovarium axis in dairy cows. There are limited publications about the ZEN effects on dairy cows' reproduction. More research is needed to elucidate the ZEN effects on reproduction.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

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