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Research Article

The antibiotic susceptibilities and microorganisms isolated from urinary tract infections of dogs

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This study was presented in 2. International Veterinary Medicine Students Scientific Research Congress (IYRSC 2020), 17-19 December, 2020, Burdur, TÜRKİYE and 22nd International Veterinary Medicine Student Scientific Research Congress, 8-9 May, 2021, Online, İstanbul, TÜRKİYE.

ABSTRACT

In this study, we aimed to isolate microorganisms from urine samples taken from dogs with urinary system infection and to determine an antibiotic susceptibility. For this purpose, urine samples taken from 30 dogs by cystocentesis were inoculated on Blood Agar, MacConkey Agar and Sabouraud's Dextrose Agar. The isolated microorganisms were identified by conventional microbiological methods. In this study, while 19 bacteria were isolated from 15 dogs (63.33%), no isolation was detected within the mycological culture. The most isolated bacteria were Escherichia coli (26.32%) and coagulase negative Staphylococci (26.32%). The isolates were susceptible 84.2% to ceftriaxone, 78.9% to enrofloxacine, 73.7% to ciprofloxacin, gentamicin and trimethoprim + sulfamethoxazole, 73.7% to amoxicillin-clavulanic acid and cefixime, 57.9% to cephalexin, 52.6% to oxytetracycline and 47.4% to ceftiofur. We concluded that Escherichia coli and coagulase negative Staphylococci were the most common reason for urinary tract infections in dogs and due to the differences to antibiotic of the bacterial isolates that antibiotic susceptibility tests is necessary for treatment.

INTRODUCTION

Urinary tract infections (UTI) are commonly seen in dogs. Some drugs, gravels, bladder neoplasms and problems concerning neural system are among the predisposed factors. UTI caused by microorganisms are commonly seen in dogs and almost 14% of dogs have these infections all their lives. *Escherichia coli* is the most frequently isolated agent and might exist by itself or together with other bacteria (Thompson et al., 2011). During the studies carried out in Canada, Turkey and Italy, it was stated that *E. coli* had been the most frequently isolated microorganisms from urinary tract infections of dogs and other Gram-negative and Gram-positive bacteria might also be isolated (Kalınbacak et al., 2004; Papini et al., 2006; Ball et al., 2008). The other bacteria isolated from UTI of dogs are *Proteus* spp., *Staphylococcus* spp., *Streptococcus* spp., *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., *Enterococcus* spp. and *Enterobacter* spp. (Ball et al., 2008). Notwithstanding quite rarely, *Acinetobacter* types can also be isolated (Sığırcı et al., 2012). Although fungi and yeast are rarely isolated from urinary tract infections of cats and dogs, *Candida* spp. is the most frequently isolated yeast. *Rhodotorula mucilaginosa* and *Cryptococcus neoformans* are the opportunist pathogens and might cause urinary tract infections (Pressler et al., 2003; Jin et al., 2005).

The most common symptoms of UTI in dogs are thamuria, dysuria, stanguria and haematuria (Byron, 2019). UTI is usually noticed by chance in cats and dogs. Clinical symptoms appear depending on the amount and virulence of the pathogen, presence of predisposed factors, response of the body to the infection and the area and duration of the infection (Bartges, 2004). UTI is seen more in female dogs than male ones. *E. coli* isolation was able to be carried out from 58% of UTI in dogs while this rate was detected 55% in male dogs (Norris et al., 2000). Treatment of UTI is generally carried out with antibiotics.

Bacteriological culture and antibiotics susceptibility tests are not always performed during these infections, yet the treatment is immediately started with antibiotics. The most crucial problem in treatment is the resistance against antibiotics depending.

The aim in this study was to determine the microorganisms causing UTI in dogs and to detect the susceptibilities of the isolated bacteria to antibiotics.

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MATERIALS and METHODS

Samples

From October 2020 to September 2021, the urine samples were collected from 30 dogs with suscepted UTI by cystocentesis. The animals used in the study were not made previously antimicrobial treatment. The urine samples were sent to the Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Microbiology for bacteriological and mycological examination.

This study was conducted within the scope of the Board decision of Burdur Mehmet Akif Ersoy University Rectorate Animal Experiments Local Ethics Committee dated August 26, 2020 and numbered 668.

Bacteriological and Mycological Examinations

The urine samples were centrifuged at 3000 rpm for 20 minutes and the supernatant was taken out from the urine, and the remaining sediment was used for bacteriological culture. Samples were inoculated onto Blood Agar (Oxoid, Hamshire, UK) with 5% sheep blood, MacConkey Agar (Oxoid, Hamshire, UK) and Sabouraud's Dextrose Agar (SDA, Oxoid, Hamshire, UK) plates. The petris were incubated for 24 hours at 37ºC in an aerobic environment, except for SDA. The samples were inoculated on two SDA petris. One petri was incubated at 25 ˚C and the other at 37 ºC for 7-10 days. The colonies were identified by conventional microbiological methods.

Antibiotic Sensitivity Tests

Disk diffusion method was performed according to Clinical and Laboratory Standards Institute (CLSI) standards (CLSI 2013). For this purpose, 0,1 mL of the bacteria suspensions adjusted according to the turbidity of the McFarland 0.5 standard suspension was taken and inoculated on Muller-Hinton Agar (Oxoid, Hamshire, UK). In this study, amoxicillin- clavulanic acid (30µg, Oxoid, Hamshire, UK), cephalexin (30µg, Oxoid, Hamshire, UK), ciprofloxacin (5µg, Oxoid, Hamshire, UK), enrofloxacin (5µg, Oxoid, Hamshire, UK), gentamicin (10µg, Oxoid, Hamshire, UK), trimethoprim/sulfamethoxazole (25µg, Oxoid, Hamshire, UK), oxytetracycline (30 µg, Oxoid, Hamshire, UK), ceftiofur (30µg, Oxoid, Hamshire, UK), ceftriaxone (5µg, Oxoid, Hamshire, UK), cefixime (30µg, Oxoid, Hamshire, UK) disks was used in antibiotic susceptibility test.

RESULTS

In this study, while 19 (63.33%) bacteria were isolated from 15 dogs, no growth could be detected in mycological culture. One bacterial agent was isolated from urine samples of 12 dogs while more than one bacteria was obtained from 3 dogs. Gram-negative bacteria were constituted 57.89% of the isolated bacteria and Gram-positive bacteria was 42.1%. *E. coli* (26.32%) and CNS (26.32%) were the most frequently isolated bacteria. Other bacteria isolated from urine samples were 15.79% *Streptococcus* spp., 10.53% *Enterobacter* spp., 5.26% *Aeromonas* spp., 5.26% *Citrobacter* spp., 5.26% *Proteus* spp. and 5.26% *P. aeruginosa* (Table 1).

Table 1. Bacterial isolates identified in urinary tract infections of dogs.

Bacteria	n	%
E. coli	5	26.32
CNS	5	26.32
Streptococcus spp.	3	15.79
Enterobacter spp.	2	10.53
Aeromonas spp.	1	5.26
Citrobacter spp.	1	5.26
Proteus spp.	1	5.26
P. aeruginosa	1	5.26
Total	19	100

The isolates were found susceptible to ceftriaxon 84.2 %, to enrofloxacin 78.9 %, to ciprofloxacin, gentamicin and trimethoprim + sulfamethoxazole 73.7 %, to amoxicillin-clavulanic acid and cefixime 73.7 %, to cephalexin 57.9 %, to oxytetracycline 52.6 % and to ceftiofur 47.4 % (Table 2). All *E. coli* isolates were found susceptible to amoxycillin clavulonic acid, cefixime and ceftriaxon. Four (80%) of the *E. coli* isolates were susceptible to amoxicillin clavulonic acid, ceftriaxon, enrofloxacin and trimethoprim sulfamethoxazole. In addition, CNS isolates were detected susceptible to ciprofloxacin, cefalexin, ceftiofur and oxytetracycline. All *Streptococcus* spp. isolates were susceptible to amoxicillin clavulonic acid, cefixim, ceftriaxon, sefalexin and ceftiofur. In this study, *Citrobacter* spp. isolate was found susceptible against to all tested antibiotics (Table 2).

DISCUSSION

Urinary tract infection is one of the most frequent infections seen in cats and dogs. Though many factors cause these infections, microorganisms are the most crucial reason. In urinary tract infections of dogs, Gram-negative bacteria are reported to have been isolated more than Gram-positive ones (Kogika et al., 1995; Çetin et al., 2003; Yu et al., 2020). Although *E. coli* was reported to have been the most frequently isolated agent from UTI of dogs, Gram-negative bacteria such as *Klebsiella* spp, *Proteus* spp., *Pseudomonas* spp. and Gram-positive ones such as *Staphylococcus* spp., *Streptococcus* spp., *Corynebac-*

Table 2. Antibiotic susceptibility of bacteria isolated from urinary tract infection of dogs.

Bacteria Isolated	Antimicrobial agents																			
	AMC		CFM		CIP		CL		CN		CRO		ENR		FUR		T		SXT	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
E. coli (n=5)	5	100	5	100	4	80	4	80	4	80	5	100	4	80	1	20	3	60	4	80
CNS (n=5)	4	80	2	40	3	60	3	60	5	100	4	80	4	80	3	60	3	60	4	80
Streptococcus spp. (n=3)	3	100	3	100	2	66.7	3	100	1	33.3	3	100	2	66.7	3	100	2	66.7	2	66.7
Enterobacter spp. (n=2)	0	0	1	50	1	50	0	0	1	50	1	50	1	50	0	0	0	0	1	50
Aeromonas spp. (n=1)	0	0	0	0	1	100	0	0	1	100	1	100	1	100	0	0	1	100	1	100
Citrobacter spp. (n=1)	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100
Proteus spp. (n=1)	0	0	1	100	1	100	0	0	0	0	1	100	1	100	1	100	0	0	1	100
P. aeruginosa (n=1)	0	0	0	0	1	100	0	0	1	100	0	0	1	100	0	0	0	0	0	0
Total (n=19)	13	68.4	13	68.4	14	73.7	11	57.9	14	73.7	16	84.2	15	78.9	9	47.4	10	52.6	14	73.7

AMC: Amoxicillin- clavulanic acid, CFM: Cefixime, CIP: Ciprofloxacin, CL: Cephalexin, CN: Gentamicin, CRO: Ceftriaxone, ENR: Enrofloxacin, FUR: Ceftiofur, T: Oxytetracycline, SXT: Trimethoprim/Sulphamethoxanole.

terium spp. could also be isolated (Cohn et al., 2003; Çetin et al., 2003; Papini et al., 2006; Sığırcı et al., 2012; Wong et al., 2015; Harihan et al., 2016; Marques et al., 2016; Bağcıgil et al., 2018; Yu et al., 2020; Vercelli et al., 2021). Kogika et al. (1995) stated that Gram-negative bacteria were the most common ones isolated from UTI of dogs, the primary isolated agent was *E. coli* (35.3%) and *Staphylococcus* spp. (23.5%) and other bacteria were isolated at various rates. Çetin et al. (2003) reported that 51 bacteria were isolated from 100 dogs with UTI, 52.94% of these bacteria were Gram-negative and the most frequently isolated bacteria was *E. coli* (23.52%). Adsanychan et al. (2019) stated that Gram-negative bacteria were the most frequently isolated ones from UTI of dogs. In this study, researchers found *Staphylococcus intermedius* as the most commonly isolated agent and *Proteus mirabilis* and *E. coli* followed respectively. Yu et al. (2020) stated that they isolated 129 bacteria from 103 out of 336 urine samples they collected from dogs in China and 33.3% of Gram-negative bacteria were *E. coli*, 12.4% *Klebsiella* spp. and 6.2% *Pseudomonas* spp. In the presented study, 7 different bacteria were isolated from UTI of dogs, *E. coli* and KNS were the most commonly isolated bacteria and *E. coli* was the most frequently isolated Gram-negative bacteria (57.89%). The obtained results in the presented study support the studies (Papini et al., 2006; Wong et al., 2015; Harihan et al., 2016; Marques et al., 2016) also stating *E. coli* as the most commonly isolated bacteria. Likewise in this study, *Klebsiella* spp., *P. aeruginosa* and *Citrobacter* spp. were isolated from urine samples and these results supported many studies stating that many bacteria might cause UTI (Cohn et al., 2003; Papini et al., 2006; Sığırcı et al., 2012; Wong et al., 2015; Harihan et al., 2016; Marques et al., 2016). Besides, some researchers (Punia et al., 2018; Adsanychan et al., 2019) reported that Gram-positive bacteria, especially *Streptococcus* and *Staphylococcus,* could be isolated more than *E. coli* in UTI of dogs. In the presented and reported studies, differences in isolation rates of bacteria might originated from geographical and climatic conditions and resistance to antibiotics due to antibiotics use (Wong et al., 2015).

In this study, *in vitro* susceptibility of the bacteria isolated from UTI of dogs was searched to ten different antibiotics. The isolates showed different susceptibilities to antibiotics in this study. The researchers (Çetin et al., 2003; Windahl et al., 2014; Bağcıgil et al., 2018; Ukah et al., 2018; Adsanychan et al., 2019; Yu et al., 2020) reported that the microorganisms were found susceptible to antibiotics at different rates. Çetin et al. (2003) stated that the most effective antibiotics in UTI of dogs were amoxycillin clavulonic acid, gentamicin, ampiciline sulbactam and enrofloxacin. Adsanychan et al. (2019) reported

that the isolates obtained from urine samples were between 66%-86% and were most susceptible to amoxycillin clavulonic acid. In this presented study, the obtained isolates were susceptible to ceftriaxone, enrofloxacin, gentamicin and ciprofloxacin but resistant to ceftiofur, oxytetracycline and cephalexin. It is thought that the differences in the susceptibilities of isolates to antibiotics might be related with the resistance developing against antibiotics used for various infections of animals.

CONCLUSION

As a result, with this study, it was revealed that Gram-negative bacteria usually caused UTI of dogs and *E. coli* and CNS were the most commonly isolated agents. Due to the differences in antibiotic susceptibilities of isolates, in UTI of dogs, antibiotics susceptibility tests were absolutely advised to be applied before treatment.

DECLARATIONS

Ethics Approval

This study was approved by Burdur Mehmet Akif Ersoy University Rectorate, Animal Experiments Local Ethics Committee (Decision No: 668/2020)

Conflict of Interest

Authors declare that there are no conflicts of interest for this study.

Consent for Publication

Not applicable

Author contribution

Idea, concept and design: DÖ, ES

Data and analysis: ES, DÖ

Drafting of the manuscript: DÖ, ES

Critical review: DÖ, ES

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Research Article

The use of snail slime collected by sustainable methods in cosmetic creams

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ABSTRACT

There are many snail slime (secretion) creams in the cosmetic industry. Many of them are formulated with herbal or synthetic allantoin when the cream ingredients are read. However, natural snail secretion also contains many bioactive components (glycoproteins, fatty acids, polyphenols, vitamins, etc.) other than allantoin. For this reason, cosmetic effectiveness decreases in products that do not contain natural snail secretion. It is not easy to reach the natural snail slime cream due to the limited collection of secretions from the appropriate snail species and limited sustainable methods. While the secretions are collected from snails by traditional methods (exposure to stresses such as salt and electricity application), animals are usually harmed or killed. If the method used for secretion collection is not suitable, animals are exposed to stress and release skin irritants. In addition, the secretion of each snail species does not have the suitable ingredients in the production of cosmetics. In this study, various essential oils and ozone were sprayed with cold steam into the porous chamber containing the snails in a specially designed instrument. Secretion was collected, from live snails (Helix aspersa) suitable for use in cosmetics, without being exposed to stress. Then the cream was formulated with this secretion. Microbiological and physicochemical analyzes were made in the cream produced. As the results; secretion containing important bioactive components was obtained from the appropriate snail species with sustainable methods, and the analysis results showed that the cream formulated with the addition of the secretion was qualified and suitable for use.

INTRODUCTION

Although the skin is defined as the outer surface of the human body, it is also categorized as an organ in the human body. Aging of the skin occurs with processes in which both internal and external factors play a role. Internal skin aging is induced by oxidative cellular metabolism from reactive oxygen species (ROS). External aging is related to air pollution, smoking, etc. Although it is dependent on exposure, it also occurs in the form of photo-aging. While external factors cause the skin to be dry, thin or thick-skinned, it causes wrinkles to be superficial or deep (Puizina-Ivi´c, 2008; Khavkin and Ellis, 2011). The term "nutricosmeceutical" is derived from combining the terms "nutraceutical" and "cosmeceutical". This term is used by the health and cosmetic industry to describe supplements, functional foods and beverages containing active ingredients that can enhance beauty and health (Kligman, 1993). With the improvement of economic conditions in the world and the increase in the elderly population, the nutricosmetic market is expected to show a 5.0% growth rate (CAGR) from 2017 to 2025 and this growth is estimated to be 7.93 billion USD by 2025. The nutricosmetic market is segmented by type of application (skin care, hair care, nail care) and region of sale. As the average age increases in Europe, it is expected that more cosmetics will be sold for the elderly. In the US region, herbal supplements are expected to be sold (Market, 2017).

In natural raw materials, new natural components have gained importance in the cosmetics industry with the 'green movement' that started in Europe at the end of 1960, and plant, marine, mineral, etc. originated and even organic cosmetic raw materials, mixtures and finished products have quickly found their place in the market (Grossman, 2005). The demand for natural cosmetic raw materials has increased the share of cosmetics in the global market. In addition to being a common food consumed by millions of people worldwide, snails are also a source of secondary metabolites used in cosmetics and medicine (Jess, 1998; Murphy, 2001). *Helix* members, an important snail genus in cosmetics, have a wide distribution including the south and east of Europe, Anatolia and the Caucasus, Iran and Jordan as their natural distribution area. The secretions of snails belonging to the genus *Helix* have been widely studied and described (Conte, 2016). Snail slime (*Helix aspersa*) is a mixture of active compounds such as proteins, glycoproteins, glycosaminoglycans, fatty acids, polyphenols, vitamins, glycolic acid, allantoin, minerals (Gugliandolo et al., 2021; Noothuan et al., 2021; Onzo et al., 2021) and carbohydrates (Newar et al., 2015). Because of these properties, snail mucus is used in cosmetics to promote the formation of collagen, elastin and dermal components that repair signs of photo-aging and to reduce damage caused by free radicals. It is stated that snail secretions can be an alternative antibacterial source to expensive synthetic antibacterial agents used in wound treatment (Iguchi et al., 1982; Ehara et al., 2002; Etim et al., 2016). Snails like moist places because they lose a lot of water through mucus secretions. In general, 80% humidity is sufficient for snails (Gökhan, 2003).

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Plant essential oils have been used for many years for different purposes, especially in many scientific and commercial areas. At the beginning of these usage areas are cosmetics, medicine, food industry, aromatherapy and phytotherapy. Essential oils show strong expectorant (accelerating mucus flow), antimicrobial and antiviral effects when used by inhalation in a censer or a container filled with water in a closed environment. Essential oils that increase mucus secretion are seen as primarily medicinal mint and eucalyptus, rosemary, lemon, chamomile, etc. Standard methods of secretion extraction from live snails are very inadequate, both analytically and industrially. The available literature shows that most of the methods tried for secretion extraction resulted in the death of snails (Das et al., 2022). For this reason, it is very important to obtain secretions without harming the snails while they are alive and will be valuable in terms of sustainable production methods and technologies.

In this study, a special instrument was designed that allows to obtain secretions without harming the snails and this instrument was used to collect the snail secretion. The snails (*Helix aspersa maxima*) with the secretion known to have skin regenerative properties and which have been identified were obtained from Burdur Mehmet Akif Ersoy University Snail Farm. The snails taken into the porous chamber of the secretion collection device were exposed to cold steam carrying ozone and essential oils, the secretion was collected and this secretion was used in the production of snail cream. Microbiological and physicochemical (colorimetric, rheological, pH) analyzes were performed on the cream.

MATERIALS and METHODS

Snails, essential oils and ozone

Snails (*Helix aspersa maxima*), identified from the Burdur Mehmet Akif Ersoy University Snail Farm, were obtained. Lavandin essential oil was obtained by distillation from Lavandin (*Lavandula x intermedia*) collected from Burdur Mehmet Akif Ersoy University campus area. 500 g stemless dry Lavandin flowers were weighed. Then the flowers were submitted to hydro distillation with a clevenger-type apparatus. The essential oil was extracted over a period of 120 min. using a clevenger apparatus. (Aytaç, 2020). Peppermint and eucalyptus essential oils and ozone to be applied to snails are commercially available. It was aimed to increase mucus secretion with sprayed ozone, peppermint and eucalyptus oil, and to have an anti-stress effect on snails with lavandin oil with cold steam.

Designing the secretion device and collecting snail secretions

The instrument used in the world to collect snail secretion without harming the living thing and working on an industrial scale were researched and examined, and a special device was designed at laboratory scale and built by a local company. The images of the instrument are given in Figure 1. The system includes an ozone and steam generator and sprays cold steam into the snail chamber at an adjustable flow rate.

The snails provided for secretion are placed in the chamber in the instrument. Each of the essential oils was added to the cold steam generator at a concentration of 0.5 mL/L. The

Figure 1. a. Snail secretion collection device b. Snail chamber c-d. Ozone and cold steam generators and secretion collection chamber

snails were exposed to cold vapor containing ozone and essential oils for 20 minutes (Figure 2). The secretion obtained after the application was filtered through a 0.45 micrometer filter, purified from possible bacterial load and solid particles, and kept at -20°C until the end of cream production and analysis (Das et al., 2022). The snails that were alive and unharmed after the procedure were returned to the Snail Farm (Figure 3-c).

Figure 2. Treatment of snails with cold steam containing ozone and essential oil in a specially designed device

Preparation method of snail slime cream

A new cream formulation containing natural snail slime was made in the R&D laboratories of Burdur Mehmet Akif Ersoy University Cosmetics Application and Research Center (KOZAM), and the analysis/tests were carried out on this product (Figure 3-b). In the cream production process (Figure 3), which

is carried out in accordance with the cosmetics legislation, thickeners, oils, emulsifiers, moisturizers and preservatives are included in the formulation together with snail secretion. The oil phase and water phase of the creams were first mixed and heated up to 80°C, then cooled to 40 °C and mixed for another 15 minutes (Şenses, 2007). Afterwards, it was passed through a homogenizer and the cream took its final shape. The secretion rate added to the cream was determined as 1%.

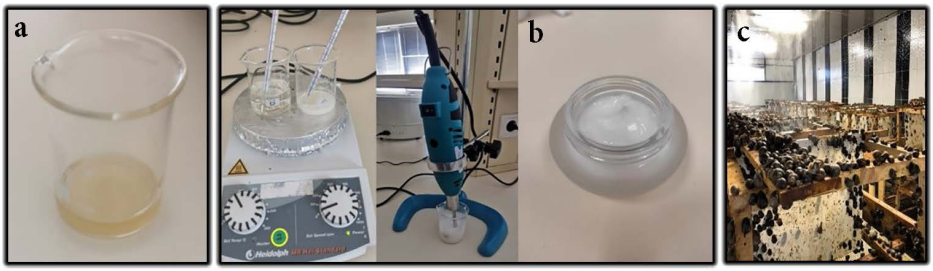

Figure 3. a. Secretion from snails b. Cream making processes (R&D laboratories of KOZAM) c. Burdur Mehmet Akif Ersoy University Snail Farm

Measurements/analysis of the snail slime cream

Rheological, colorimetric and pH measurements of the cream as well as microbiological analyzes were made. Measurements and analyzes were performed in three replications of the cream on "the 1st day (sample 1), the 30th day (sample 2) and the 60th day (sample 3)". The cream was stored in a frosted glass cream jar at room temperature (20°C±2) until the analysis was completed.

Rheology is a measure of the resistance of a fluid to deform under surface tension. It can also be defined as the internal resistance of the fluid to flow. Rheological measurement results are given in Table 1. Brookfield RV/DV-II+Pro Extra Viscometer Device (Brookfield Engineering Laboratories, Inc., Middleboro MA, USA), Spindle No: RV 6 was used for the measurements. Analysis was carried out in a 50 mL falcon tube (plastic tube with a centrifugal cap) at room temperature (Bovey, 1965; Erbil, 2000).

Konika Minolta (Chroma Meter CR-400/410) was used for colorimetric (CIELab) measurements. L*, a* and b* measurements were made by calibrating the color device with a white plate and using the D65/10 light source in 3 repetitions. The whiteness value of the samples was determined by Sarbon et al. (2015)'s method is calculated according to the formula below.

$BI = 100 - [(100 - L^{\square})^{2} + a^{\square 2} + b^{\square 2}]^{1/2}$ (Metin, 2020).

Mettler Toledo brand and S20K model pH meters were used for pH measurements of creams. Measurements were made with a calibrated pH meter. For each formulation, the measurement was repeated 3 times and the average was recorded (Oğuz, 2019).

"Total mesophilic aerobic bacteria count" and "total yeast-mold count" analyzes were performed on the cream sample. In the cream sample, 1/10 dilution was carried out in suspension containing 0.09% NaCl. For total mesophilic aerobic bacteria count, PCA (Plate Count Agar) and for Yeast-Mold count PDA (Patato Dextrose Agar) agar medium, the cream sample diluted with 3 repetitions was inoculated with 1 mL drop and inoculated as a smear (Gök et al., 2019). Incubation was carried out at 35°C for 48 hours for total mesophilic aerobic bacteria count, and for 72 hours at 25°C for yeast-mold counting. Colony count was performed after incubation.

RESULTS

In all parameters, depending on the day the snail slime cream was prepared, three results were given as the 1st day (sample 1), the 30th day (sample 2) and the 60th day (sample 3). The results of all measurements and analyzes studied in the cream formulated with snail secretion are given in Table 1-4.

The rheological results of the prepared snail cream are given in Table 1. Rheological measurements were carried out at different rpm (revolution per minute) values (10-100 rpm). When all results were examined, the first measurement was found 34200-5120 mPa.s, the 30th day as a result of 28100-4000 mPa.s and the 60th day as a result of 27834-2260 mPa.s.

Table 1. The Viscosity Measurements (RPM) of Snail Slime Cream

Sample	10	20	30	40	50	60	70	80	90	100
1	34200± 305	18400± 684	13600± 723	10700± 532	8960± 380	7800± 256	6914± 345	6150± 510	5555± 340	5120± 278
2	28100± 942	12800± 1230	9989± 441	7875± 761	6733± 804	6294± 587	7062± 720	5117± 404	5393± 284	4000± 524
3	27834± 2753	10284± 1411	4100± 295	3500± 349	4980± 180	4544± 323	4550± 959	3339± 330	2576± 33	2260± 187

The results of the colorimetric measurements of the cream are given in Table 2. The measurement results of the three

samples are close to each other, no significant change detected. L values were found very close to 100 in all samples.

Table 2. The Colorimetric Measurements of Snail Slime Cream

Sample	L*	a*	B*
1	85.61	3.37	-4.43
2	84.83	3.39	-4.41
3	84.85	3.35	-4.42

The pH results are given in Table 3. When the results of the cream pH measurements were examined, no significant difference was found in all samples. pH values were measured between 5.43-5.49.

Table 3. The pH Measurements of Snail Slime Cream

Sample	pH
1	5.46
2	5.43
3	5.49

The results of microbial analysis in the cream are given in Table 4. As a result of the analysis, no contamination was detected in 3 samples.

metic manufacturers generally use herbal or synthetic allantoin instead of snail secretion and market it only as a snail slime cream. Natural snail secretion contains important bioactive components, especially allantoin, but if the method used for secretion collection is not suitable, animals experience stress and secrete skin irritants. In addition, not all snail species are suitable for cosmetic use. When the secretion collected by sustainable methods, using snail species suitable for cosmetics, is added to cosmetic formulations, it increases the added value and quality of the product considerably. Creams are one of the most frequently used products among cosmetics. Creams, in the simplest terms, are systems called emulsions, which are formed as a result of the dispersion of two immiscible liquids such as water and oil in one another with the help of a third component, an emulsifier. Apart from these three basic ingredients, in creams; There are lubricants, thickeners or viscosity increasers, auxiliary emulsifiers, moisturizers, preservatives and essences (Şenses, 2007).

In this research, secretion was collected from *Helix aspersa maxima*, which is known to be rich in bioactive components (Gugliandolo et al., 2021; Noothuan et al., 2021; Onzo et al., 2021), with sustainable methods, without harming the living thing, and was used in the production of snail slime cream. Rheological, colorimetric and pH measurements of the produced cream as well as microbiological analyzes were made (Table 1-4).

Table 4. The Antimicrobial Activity of Snail Slime Cream

Sample	Total Mesophilic Aerobic Bacteria Count	Yeast-Mold Count
1	- (Not found)	- (Not found)
2	- (Not found)	- (Not found)
3	- (Not found)	- (Not found)

DISCUSSION

The word cosmetics in the Cosmetics Regulation published by the Ministry of Health in 2005; "*It is prepared to be applied to the outer parts of the human body, the epidermis, nails, hair, lips and external genitalia, or the teeth and oral mucosa, for the sole or main purpose of cleaning these parts, giving them fragrance, changing their appearance, protecting them, keeping them in good condition or it refers to all substances or mixtures of substances with the purpose of correcting their odor*" (Ministry of Health, 2005).

The use of cosmetics is one of the indispensable routines of people of all ages, regardless of gender. This increases the economic growth and mobility that falls on the share of the cosmetics industry (Market, 2017). The demand for cosmetics that include natural raw materials and important bioactive components in its formulation is increasing in the industry. Snail slime cream, serum, etc. cosmetic products are also in high demand in this context. However, reaching the natural snail secretion is not sustainable as it harms the living thing. However, reaching the natural snail secretion is limited because it harms the snails and it is difficult to obtain it continuously and sustainably. For this reason, some of the cos-

Brookfield viscometer has limited sensitivity; The change in the behavior of the Non-Newtonian fluid depending on the temperature change or temperature change creates a constant difference in the viscosity measurement. Measurements should be made with the same spindle at constant temperature and constant rotational speed (Bovey, 1965; Erbil 2000). In the prepared cream; In the measurements made with the same spindle at constant temperature, it was determined that the rheology of the snail cream decreased at a statistically insignificant level from the day it was produced to the 60th day (Table 1). However, it was observed that the cream was spreadable at all three measurement times, including the rheological values after 60 days (Kwak, 2015). It is thought that the decrease in rheology may be due to time-dependent deterioration and segregation under stabilization conditions, and this situation is in parallel with similar literature (Oğuz, 2019). It is anticipated that this situation can be prevented by storing the cream at +4°C, which has already been stored at room temperature.

Whiteness value is an important criterion for industrial applications. In cosmetic products/creams, it is of great importance not to change the color of the formulation and to

obtain the light color that is generally preferred by consumers (Metin, 2020). CIE L*, a*, b* The color system contains three coordinates. These; (L*) brightness (0,black ;100,white), (a*) red to green (+a,red; -a,green), and (b*) yellow to blue (+b,yellow; b,blue) and (ıy*) represents the jaundice index (Snell et al., 2002). Accordingly, L* value ranges from 0 (black) to 100 (white), a* value indicates red-green and b* value indicates yellow-blue scale (Üren, 1999). In the study, the formula given in the method was applied and color measurements depending on the day (1st, 30th and 60th days) were determined. Accordingly, it was calculated as 84.6, 83.8 and 83.9, respectively, and the color is quite white (Table 2). Accordingly, the color change in the cream on a daily basis (1st, 30th and 60th days) is negligible. The whiteness value of the cream meets the expectation in all three measurements.

The pH of the skin is normally acidic and the pH value varies between 4.1-5.8 (Proksch, 2018; Sethi et al., 2016). When the pH values obtained from the prepared cream were examined, no significant change was observed at the end of the 1st, 30th and 60th days, and this shows that this formulation is stable in terms of pH value. All pH measurement results were found between 5.43-5.49 (Table 3). As a result, all creams are compatible with the skin in the desired pH range.

As a last parameter, as a result of microbiological analysis performed on snail slime cream, no microorganism growth was found in the cream in 3 analyzes performed on the 1st, 30th and 60th days (Table 4).

It is thought that this is due to the fact that the snails are processed after they are cleaned with water, the ozone sprayed on the snails and the presence of peppermint-eucalyptus essential oils containing menthol, which are known to have antimicrobial effects (Bocci, 2006; Turcheniuk et al., 2015), the preservative added to the cream formulation does not allow contamination, and the cream formulation is prepared in an aseptic environment.

CONCLUSION

In the research study, snail slime cream was prepared with sustainable methods and without harming the snails, then rheological, colorimetric, pH measurements and microbial analyzes were made in the cream at the end of the 1st, 30th and 60th days. When the results were evaluated, it was seen that the snail slime cream produced was compatible with the skin acidity. Color and spreadability meet user expectations. In addition, it was concluded that the absence of microbial activity in the product for 60 days is important for product safety. Furthermore, due to the snail secretion rich in bioactive components in the cream formulation, it has an important care-repair and renewal feature. It may be an important formulation that can be put on the market after more detailed and long-term studies are done.

DECLARATIONS

Ethics Approval

Since the snails, which are the study material, are invertebrates, the approval of the ethics committee is not required according to the regulation on the working principles and procedures of the animal experiments ethics committees published in the official newspaper dated 15.02.2014 and numbered 28914.

Conflict of Interest

The author declare that they have no conflict of interests.

Consent for Publication

Not applicable

Author contribution

Idea, concept and design: ACT

Data collection and analysis: ACT

Drafting of the manuscript: ACT

Critical review: ACT

Data Availability

The data used to prepare this manuscript are available from the corresponding author when requested.

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Research Article

Bacterial agent isolation from calves with arthritis and antibiotic susceptibility of isolates

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ABSTRACT

Septic arthritis is frequently encountered in neonatal calves, and it causes significant economic losses as a result of high mortality rates. The main objective of this study was to isolate and molecularly identify the common bacterial agents, Erysipelothrix rhusiopathiae, Trueperella pyogenes, Mycoplasmopsis bovis, Staphylococcus aureus, and Escherichia coli, that cause arthritis in calves, and to contribute to the selection of appropriate antibiotics in the treatment of the disease caused by these agents by determining the antibiotic susceptibility of the isolated species. This study bacteriologically examined synovial fluid samples taken from 65 calves 0–3 months old that were brought to the animal hospital clinics of the Faculty of Veterinary Medicine of Kafkas University between 2018 and 2020. E. rhusiopathiae, S. aureus, and M. bovis were each isolated in a single sample, separately. T. pyogenes was isolated in two samples, and T. pyogenes and E. coli were detected together in one sample. Results of direct PCR from the synovial fluid samples found T. pyogenes DNA in 18 samples, S. aureus DNA in 8 samples, and T. pyogenes and S. aureus DNA together in 3 samples. The results of the antibiogram test found that E. coli was sensitive to tetracycline, macrolide, lincosamide, phenicol, fluoroquinolone, cephalosporin and vancomycin group antibiotics; T. pyogenes isolates were sensitive to quinolone, linlozamide, beta-lactam, fluoroquinolone, cephalosporin, sulfonamide and vancomycin group antibiotics; S. aureus was sensitive to aminoglycoside, quinolone, fluoroquinolone, cephalosporin, sulfonamide and vancomycin group antibiotics; E. rhusiopathiae was sensitive to quinolone, beta-lactam, sulfonamide and cephalosporin; M. bovis was sensitive to macrolide, lincosamide, phenicol and fluoroquinolone group antibiotics.

INTRODUCTION

In cattle breeding, arthritis is among the diseases that occur mostly in dairy cattle (Gharagozlou et al., 2004; Sababoglu et al., 2018) and in calves, and it causes significant economic losses (Yurdakul & Apaydın Yıldırım, 2019). Neonates appear to be most at risk for septic arthritis which can be life threatening (Arican et al., 2022). Arthritis is a disease that shows signs of fever, pain, swelling, and lameness at different levels in calves (Yurdakul, 2018; Sutradhar et al., 2019). Arthritis shows itself as acute-chronic aseptic-septic joint inflammation, which can affect one, several, or all of the components of the joint (Oktem & Anteplioglu, 1967; Bailey, 1985; Ozaydin, 1990; Arican et al., 1998; Bumin et al., 2001). Especially, septic arthritis is occasionally diagnosed in calves because the calf is at high risk of developing septic arthritis at birth due to umbilical infection and consumption of contaminated milk (Tsuka et al., 2020).

Arthritis develops either following traumatic external effects on the joint (such as luxation, distortion, or overstrain) or transmission to the direct joint or neighbor tissues of infectious factors together with traumatic effects, also with the location of these factors in the joints in a hematogenous way (Jesse et al., 2017; Arican et al., 2022) as the result of diseases such as septicemia, omphalitis, and pneumonia (Arican et al., 1998; Samsar & Akın, 2000).

Factors in the formation of diseases that cause the emergence of infectious arthritis in newborns include failure to pay attention to birth hygiene, insufficient or no postpartum umbilical hygiene, inadequate colostrum, and defective background and heredity, which constitute significant predisposition (Cihan et al., 2002). As such, morbidity and mortality rates increase in calves in the neonatal period. The prevalence of arthritis on a farm is of absolute importance. In this regard, septic arthritis caused by different types of microorganisms (bacteria, viruses, or fungi), which is the most common cause of arthritis and particularly harms the individual production of the animal, must be diagnosed and treated in the early period to control the infection and limit its degenerative effect on the articular cartilage. It is important. Thus, in addition to a successful treatment, the chance of restoring the function of the joint may increase. However, many animals are sent to slaughter every year because the treatment of the disease is long and expensive, and sick animals show poor performance (Gokhan & Ozturk, 2016). Arthritis is of great importance in terms of its prevalence, treatment, and economy. In the formation of these diseases that provide the emergence of infectious arthritis in newborns, insufficient colostrum, and factors such as uneven ground and heredity, which constitute significant predispositions, besides the factors such as not taking care of birth hygiene, and insufficient or no postpartum umbilical hygiene are effective (Cihan et al., 2002). Therefore,

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morbidity and mortality rates increase in calves in the neonatal period. Regardless, the incidence and prevalence of arthritis are significant for farms. In this regard, treatment is valuable in the early period to limit its degenerative effect on the articular cartilage and to take control of infection. Thus, together with treatment, the chance of recovery of the function of the joint may increase. However, many animals are sent to slaughter every year because the treatment of the disease is long and expensive, and sick animals show poor performance (Gokhan & Ozturk, 2016). Arthritis is important in prevalence, treatment, and economics (Cihan et al., 2002).

During arthritis, synovial fluid is usually clear or slightly pale yellow and viscous, with the appearance of egg whites containing protein, becomes abnormal, and includes numerous leukocytes and microbial pathogens (Rohde et al., 2000).

Many methods are used in the diagnosis of arthritis. Among the basic diagnostic techniques are anamnesis, clinical examination, radiological, and arthroscopic examinations (Desrochers, 2004a), blood analysis (Jesse et al., 2017). Also, examination of synovial fluid is also one of the important ways to detect joint disorders that occur with the progression of various diseases, including arthritis (Tsuka et al., 2020). Clinical findings play a significant role in the initial diagnosis (Pritchard et al., 1979; Power & Rebhun, 1983; Desrochers, 2004a). However, it has been reported that the analysis of the synovial fluid taken from the suspected joint will not be sufficient unless microbiological culture is performed (Watkins & Sharp, 1998; Ismail et al., 2007; Sababoglu et al., 2018). On the other hand, it has also been reported that the causative agent cannot always be isolated from synovial fluid samples taken from animals that are thought to be clinically infected, and a cultural poll is necessary to differentiate between infectious and non-infectious arthritis (Madison et al. 1991; Rohde et al., 2000), *T. pyogenes* (Gharagozlou et al., 2004; Ismail et al., 2007), *Mycoplasma* spp. (Stalheim & Stone, 1975; Gharagozlou et al., 2004), coagulase-negative *Staphylococcus* (CNS) (Gharagozlou et al., 2004), *S. aureus* (Ismail et al., 2007; Dogan et al., 2016), *Streptococcus* sp. (Ismail et al., 2007), *E. coli* (Goodarzi et al., 2015; Dogan et al., 2016), and *Erysipelothrix* spp. (Kallo et al. 1997) are among the most common bacterial agents encountered in arthritis cases in calves.

The treatment of septic arthritis mainly involves joint lavage with antibiotics and anti-inflammatory approaches, but may also require surgical interventions. Only hyperacute cases of septic arthritis can be treated conservatively, whereas chronic cases require surgery (Jost & Sickinger, 2021).

Different antimicrobial drugs are used as monotherapy or in combination with intra-articular and/or parenteral injection to treat septic arthritis (Beccati et al., 2015), including b-lactams, aminoglycosides, sulfonamides, fluoroquinolones, macrolides. rapamycin and amphenicols (Hall et al., 2012). Broad-spectrum antimicrobials and those with high intra-articular concentrations should be preferred (Haerdi-Landerer et al., 2010).

Multidrug resistance of bacterial species is an urgent global problem that falls under the concept of "One Health" (Motta et al., 2017) and poses a major threat to the control and treatment strategies of many infectious diseases affecting pets (Giguère et al., 2015). Studies on this subject are limited in number (Morton, 2005; Carstanjen et al., 2010) However, to date, the extent of drug resistance or the degree of multidrug resistance (MDR) of pathogens isolated from septic arthritis cases has been unclear or unrecognized (Motta et al., 2017).

The scope of this study is to determine the common bacterial pathogens, *E. rhusiopathiae, T. pyogenes*, *M. bovis, S. aureus*, and *E. coli,* causing infectious arthritis in calves in the Kars province and the antibiotic susceptibility of these agents by evaluating the bacteriological culture results obtained from synovial fluid samples with suspicion of arthritis that were sent to Animal Hospital Clinics and Microbiology Department Laboratory of Faculty of Veterinary Medicine, Kafkas University between 2018 and 2020 years.

MATERIALS and METHODS

Ethical statement

Ethical approvals of the study were obtained from and the Kafkas University Animal Experiments Local Ethics Committee (KAU-HADYEK/2017-089).

Reference Strains

Trueperella pyogenes and *M. bovis* reference strains that were kindly provided by Department of Microbiology, Faculty of Veterinary Medicine, Selçuk University and Department of Microbiology, Faculty of Veterinary Medicine, Atatürk University, respectively and *S. aureus* ATCC 25923 and *E. rhusiopathiae* ATCC 19414 were used as positive controls throughout the study.

Study Areas and Sampling

The animal material of the study consisted of 65 calves of different ages, breeds, sex, history of lameness, and joint swelling that were brought to the Animal Hospital Clinics of the Faculty of Veterinary Medicine of Kafkas University from the center of Kars and its surrounding villages between 2018 and 2020.

Following the anamnesis of the calves, clinical examinations were done systematically through inspection, palpation, direct radiography, arthrocentesis, and macroscopic examination of the joint fluid.

Clinical Examination

During the clinical examination, the extremities with complaints of lameness were checked in detail. It was determined whether the arthritis was monoarthritis or polyarthritis (Table 1). A two-way (M/L, A/P) radiographic image of the involved joint was taken. No treatment was recommended for calves with polyarthritis, severe cartilage damage, or arthrosis. Cases with a penetrating wound to the joint or fistula were not included in the study.

Collecting Synovial Fluid Samples

The macroscopic state of the synovial fluid (the presence of fibrin clots, turbidity, and viscosity) was evaluated and re-

corded (Table 1). After shaving the related joint, antisepsis was applied to the area with povidone-iodine. It was prepared for arthrocentesis by limiting it with sterile covers. After asepsis, the dorsomedial pouch of the involved joint was entered with a size 20 sterile pink cannula. The content was aspirated, and 2 mL of synovial fluid was drawn with a 2.5 mL syringe as examination material (Dogan et al., 2016).

The samples were sent to the microbiology laboratories of the faculty within 10 minutes and at 4℃ and examined using cultural methods.

Microbiological Examination

Bacteriological Culture Conditions and Agent Isolation

Synovial fluid samples were sown into 5% sheep blood agar (Oxoid, CM0271), EMB agar (Oxoid, CM0069), and Mac Conkey agar (Difco, 212123) mediums for isolation of *Trueperella* spp., *Staphylococcus* spp., *E. coli* and *Erysipelothrix* spp. Petri dishes were incubated at 37°C for 24-48 hours under aerobic conditions (Quinn et al., 1994). Mycoplasma broth base (Oxoid, CM0403) was used for the isolation of *Mycoplasma* spp., and the tubes were incubated at 37°C for seven days under microaerobic conditions. After incubation, 100 µl of enrichment cultures were taken and inoculated into Mycoplasma selective agar (Oxoid, CM0401). Petri dishes were incubated under the same conditions. The mediums also contain horse serum and yeast extract as enrichment and thallium acetate and penicillin as selective components (Otlu, 1996).

Grown colonies were evaluated in both macroscopic and microscopic morphology after Gram staining. Suspicious *Mycoplasma* colonies were viewed under a light microscope at 10–40x (20-60x stereomicroscope) magnification (Sababoglu et al., 2018), and granular colonies with or without center, uniform or irregular terminations, expressed as fried eggs, were first and simple identified as possible *Mycoplasma* spp. (Otlu 1996). Absolute identification of suspected *Mycoplasma* spp. colonies was performed by PCR-based molecular techniques. Suspected *Mycoplasma* spp. colonies for use in advanced identification techniques based on PCR were cut together with the medium with a sterile scalpel tip and put into Brucella with 20% glycerin Broth (Sigma, B3051) and stored at -20 °C. For *Erysipelothrix* spp., colony morphology, Gram staining features of the agent, reactions in the catalase test, and the H_2S production test were taken into consideration (Balootaki et al., 2017). All isolates were defined via standard bacteriological methods that determined biochemical and reproductive characteristics (Quinn et al., 1994).

DNA Extraction

The heat treatment method was used with a single colony lysis buffer solution (SCLB) for DNA extraction from the isolates obtained in the study (Marmur, 1961). A single colony was selected from the colonies grown on solid media and transferred into freshly prepared 40 µl single colony lysis buffer in 0.2 ml PCR tubes and homogenized in mixing wells. These tubes were placed in a gradient heat machine and kept at 80°C, 55°C, and + 4°C for 10 minutes each as one cycle. At the end of the period, 80 µl of nuclease-free water was added to the tubes. The tubes were centrifuged at 7.000 rpm for 2.5 min. After centrifugation, approximately 80 µl of supernatant was collected and used as template DNA. The obtained DNA extracts were stored at -20°C until they were used in the PCR process.

Polymerase Chain Reaction and Amplification

A species-specific PCR technique was applied to identify the isolates obtained in the study at the species level. The primer pairs used are presented in Table 3. PCR reactions were performed as recommended by the manufacturer. For this purpose, reaction volumes for molecular identification of *T. pyogenes, S. aureus, M. bovis,* and *E. rhusiopathiae* were performed as recommended by Ulbegi-Mohyla et al. (2010), Martineau et al. (1998), Hananeh et al. (2018), and Balootaki et al. (2017), respectively.

For *S. aureus*, PCR conditions were 96°C for 3 min, 35 cycles each at 95°C for 1 min, 55°C for 30 s, and 72°C for 3 min, with a final extension at 72°C for 4 min. The 108 bp band length was considered positive (Dakhael et al., 2016). PCR conditions for *T. pyogenes* typing were 95°C for 10 min, 35 cycles each at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 5 min. The expected product size was 704 bp (Ulbegi-Mohyla et al., 2010). Species-specific PCR conditions for *M. bovis* were applied at 94°C for 5 min, with 40 cycles each at 94°C for 45 s, 52°C for 45 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. The expected product size was 319 bp (Hananeh et al., 2018). Thermal conditions for *E. rhusiopathiae* were 95°C for 5 min, 30 cycles each at 95°C for 1 min, 54°C for 2 min, 72°C for 2 min, and a final extension at 72°C for 7 min. The 407 bp band length was considered positive (Balootaki et al., 2017).

Antibiotic Susceptibility Test

The Kirby Bauer disc diffusion method was used to determine the antibiotic susceptibility of the isolates (Bauer et al., 1966). The colonies were suspended in a saline solution of 0.9% NaCI. Turbidity was adjusted to 0.5 McFarland standard (about 10^8 CFU/mL) and used as the inoculum for the antibiotic tests. Afterward, 0.1 mL of bacterial suspension was spread on Muller Hinton agar, and antibiotic disks were placed on the agar. Twenty different antibiotic disks belonging to 11 different groups (aminoglycoside, tetracycline, quinolone, macrolide, lincosamide, phenicol, beta lactam, flouroquinolone, sulfonamide, cephalosporin, vancomycin) were used, including streptomycin (10 µg; Oxoid, UK), oxytetracycline (30 µg; Oxoid, UK), ampicillin (25 µg; Oxoid, UK), penicillin G (10 U; Oxoid, UK), erythromycin (15 µg; Oxoid, UK), lincomycin (10 µg; Oxoid, UK) (Goodarzi et al., 2015), amoxicillin + clavulanic acid (30 µg; Oxoid, UK), gentamicin (10 µg; Oxoid, UK), trimethoprim-sulfamethoxazole (25 g; Oxoid, UK), florfenicol (30 µg; Oxoid, UK) (Jost & Sickinger, 2021), neomycin (30 µg; Oxoid, UK), ciprofloxacin (5 µg; Oxoid, UK) (Balootaki et al, 2017), sulbactam + ampicillin (30 µg; Oxoid, UK) (Ayhanci et al., 2018), ofloxacin (5 µg; Oxoid, UK), cloxacillin (5 µg; Oxoid, UK), danofloxacin (5 µg; Pfizer), amoxicillin (25 µg; Oxoid, UK), cefaperazone (75 µg; Bioanalyse, UK), chloramphenicol (30 µg; Oxoid, UK), and vancomycin (30 µg;

Oxoid, UK). The plates were incubated under the necessary conditions for isolates. Evaluations were made according to Clinical and Laboratory Standards Institute (CLSI, 2020) standards. Multidrug resistance (MDR) is considered when an isolate shows simultaneous resistance to three or more groups of drugs (Motta et al., 2017).

RESULTS

Clinical Examination Results

Forty-eight cases had monoarthritis, and 17 cases had polyarthritis. Calves were 15 days to 3 months of age and included 38 males and 27 females (Table 1). Table 2 presents synovial and radiological data related to 65 calves with septic arthritis. Omphalitis was detected in any calves.

Bacterial Isolation and Identification Results

While the causative agent was isolated from 6 of the samples, the agent could not be isolated from 59 samples. As shown in Table 4, *E. rhusiopathiae, S. aureus*, and *M. bovis* were each isolated in one sample, separetely each isolated in a single sample, separately. *T. pyogenes* was isolated in 2 samples, and *T. pyogenes* and *E. coli* were detected together in 1 sample. Bacterial isolation was performed from synovial fluid samples obtained from 3 female and 3 male calves. 1 *T. pyogenes*, 1 *S. aureus* with 1 *T. pyogenes* and 1 *E. coli* were isolated from synovial fluid sam-

Table 1. Information for 65 calves with suspicion of arthritis.

Variable	Number
Breed	
Simmental	52
Crossbreed	4
Simmental hybrid	5
Brown Swiss	4
Age	
0–15 days	12
15–30 days	27
30–60 days	22
60–90 days	4
Sex	
Male	38
Female	27
Clinical Examination	
Lameness	
Present	62
Absent	3 (light-intermittent)
Number of Affected Joints	
Monoarthritis	48
Polyarthritis	17
Lameness Time	
1–5 days	42
5–10 days	17
10–30 days	4
More than 30 days	2
Colostrum Intake	
Yes	65
No	0
First Breastfeeding Hour	
30-60 min.	5
60-90 min.	6
90.120 min.	36

Table 2. Radiological and synovial findings related to calves with septic arthritis

Synovial Findings	
Color	
Clear	1
White (Pus)	49
Yellow	8
Pink-Red	7
Synovial Findings	
Viscosity	
Viscosity (+)	0
Viscosity (-)	65
Radiological Findings	
Cartilage Degeneration	
Present	13
Absent	52

Table 3. Primer pairs used in identification of isolates.

Test	Bacteria	Primer pair	Oligonucleotide sequences (5'-3')
PCR	*S. aureus*	Sa442	AATCTTTGTCGGTACACGATATTCTTCACG
		Sa442	CGTAATGAGATTTCAGTAGATAATACAACA
	M. bovis	Mb1	AAGGTACACCAGCTAACCCAG
		Mbr2	AATGAAGCTACTGATCCAAG
	T. pyogenes	plo	CGATCCCTCTGGTGTACTTGC
			GCTTGACAAAAATCTGGCGTCC
	E. rhusiopathiae	MO101	AGATGCCAT-AGAAACTGGTA
		M0102	CTGTATCCGCCATAACTA

Table 4. Agents isolated from arthritis in the calves.

Agent	Number	%
E. rhusiopathiae	1	1.54
S. aureus	1	1.54
T. pyogenes	2	3.08
E. coli	1	1.54
M. bovis	1	1.54
No isolation	59	90.76
Total	**65**	**100**

ples taken from 3 simmental female calves, 1 *T. pyogenes* from a crossbreed male calf and 1 *M. bovis* from a simmental hybrid male calf and 1 *E. rhusiopathiae* from a brown swiss male calf. Synovial fluid samples were seen clear, yellow, pink-red and white in colour via macroscopic examination and bacterial isolations were performed especially from yellow, white and pink-red coloured synovial samples.

Polymerase Chain Reaction Results

The obtained isolates were identified as *T. pyogenes, E. rhusiopathiae, S. aureus,* and *M. bovis* by species-specific PCR (Figure 1). In the results of PCR performed directly from synovial fluid samples, *T. pyogenes* and *S. aureus* DNA were detected in 18 and 8 specimens, respectively. Also, DNAs of *T. pyogenes* and *S. aureus* were found together in 3 specimens (Table 5).

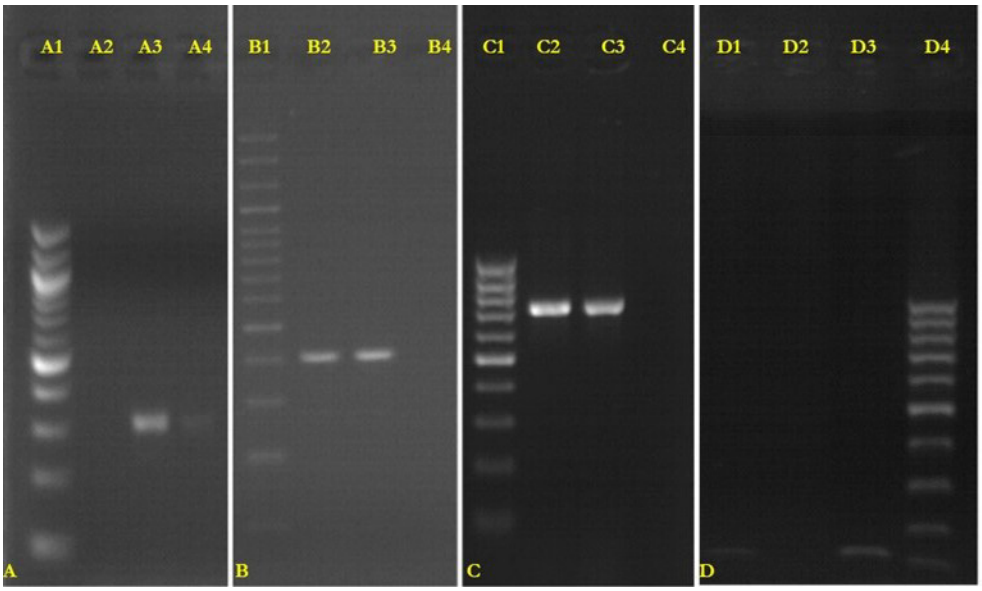

Figure 1. 1.5% agarose gel electrophoresis images of species-specific PCR products.
A: M. bovis specific PCR products (319 bp) A1: Clear Band DNA Marker 100 bp -Green (Eco Tech Biotechnology DM100) A2: Negative control A3: Positive control A4: Field strain
B: Erysipelothrix rhusiopathiae specific PCR products (407 bp) B1: GeneRuler 100 bp Plus DNA Ladder (Thermo Sci SM1153) B2: Field strain B3: Positive control B4: Negative control
C: T. pyogenes specific PCR products (704 bp) C1: GeneRuler 100 bp DNA Ladder (Thermo Scientific SM0243) C2: Field strain C3: Positive control C4: Negative control
D: S. aureus specific PCR products (108 bp) D1: Field strain D2: Negative control D3: Positive control D4: GeneRuler 100 bp DNA Ladder (Thermo Scientific SM0243)

Table 5. Bacterial agents identified by direct PCR from synovial fluid samples.

Type of sample	Identified bacterial agent	PCR positive sample number/ Total sample number	%
Synovial fluid	*T. pyogenes*	18/65	27.7
Synovial fluid	*S. aureus*	8/65	12.3
Synovial fluid	*T. pyogenes*+*S. aureus*	3/65	4.62
Total		**29/65**	**44.62**

DNA bands belonging to these agents were detected in the direct PCR test performed on the synovial fluid samples from which the agents were isolated. Of the 29 positive samples, 16 were from female and 13 were synovial fluid samples from male calves.

Antibiotic Susceptibility Test

The results obtained from the antibiotic susceptibility test were expounded according to CLSI (2020) standards. According to this, *E. rhusiopathiae* was resistant to streptomycin, erythromycin, oxytetracycline, lincomycin, neomycin, and chloramphenicol and susceptible to the other antibiotics. *E. coli* was resistant to amoxicillin-clavulanic acid, gentamicin, ampicillin-sulbactam, ciprofloxacin, and trimethoprim-sulfamethoxazole and susceptible to the other antibiotics *S. aureus* was resistant to penicillin G, oxytetracycline, erythromycin, cloxacillin, chloramphenicol, neomycin, and lincomycin and susceptible to the other antibiotics. 3 *T. pyogenes* isolates were resistant to neomycin, gentamicin, erythromycin, oxytetracycline, and chloramphenicol and susceptibile to the other antibiotics. *M. bovis* was resistant to streptomycin, ofloxacin, cloxacillin, neomycin, amoxicillin, amoxicillin, clavulanic acid, cefoperazone, penicillin G, vancomycin and trimethoprim-sulfamethoxazole and susceptible to the other antibiotics. Table 6 presents the antimicrobial resistance panels of the isolates.

At the result of this study it was seen multi drug resistance (MDR) in obtained all isolates (Table 6). *E. coli* showed resistance to three different classes of antibiotics: cephalosporin, beta-lactam and sulfonamide. *T. pyogenes* was resistant to 4 antibiotic classes including aminoglycoside, tetracycline, macrolide and phenicol; *S. aureus* was resistant to 6 antibiotic classes including aminoglycoside, tetracycline, macrolide, lincosamide, beta-lactam and amphenicol; *E. rhusiopathiae* exhibited multidrug resistance to 6 antibiotic classes including aminoglycoside, tetracycline, macrolide, lincosamide, phenicol and vanco-

Table 6. Antimicrobial resistance panel of isolates

Antibiotic class	Antibiotics	Isolates									
		E. coli		*T. pyogenes*		*S. aureus*		*E. rhusiopathiae*		*M. bovis*	
		S	R	S	R	S	R	S	R	S	R
Aminoglycoside	Streptomycin (S)	+		+		+			+		+
	Gentamicin (CN)		+		+	+		+		+	
	Neomycin (N)	+			+		+		+		+
Tetracycline	Oxytetracycline (OT)	+			+		+		+	+	
Quinolone	Ofloxacin (OF)	+		+		+		+			+
	Ciprofloxacin (CIP)		+	+		+		+		+	
Macrolide	Erythromycin (E)	+			+		+		+	+	
Lincosamide	Lincomycin (MY)	+		+			+		+	+	
Phenicol	Florfenicol (FFC)	+		+		+		+		+	
	Chloramphenicol (C)	+			+		+		+	+	
Beta-lactam	Penicillin G (P)	+		+			+	+			+
	Ampicillin (AMP)	+		+		+		+			+
	Sulbactam + Ampicillin (SAM)		+	+		+		+			+
	Amoxicillin (AML)	+		+		+		+			+
	Amoxicillin + Clavulanic acid (AML)		+	+		+		+			+
	Cloxacillin (OB)	+		+			+	+			+
Fluoroquinolone	Danofloxacin (D)	+		+		+		+		+	
Cephalosporin	Cefaperazone (CFP)	+		+		+		+			+
Sulfonamide	Trimethoprim-Sulfamethoxazole (TMP-SXT)		+	+		+		+			+
Vancomycin	Vancomycin (VA)	+		+		+			+		+

mycin; *M. bovis* exhibited multidrug resistance to 5 antibiotic classes including aminoglycoside, quinolone, cephalosporin, sulphonamide and vancomycin.

DISCUSSION

Arthritis is a serious cause of lameness in animals (Rohde et al., 2000; Jesse et al., 2017). Actually, arthritis is a welfare issue in ruminants and, if not adequately treated, can cause prolonged severe pain, reduced joint function and reduced range of movement. In addition, it can lead to altered normal joint physiology due to severe pain, which can contribute to rapid and permanent destruction of articular cartilage and bone. Furthermore, in cases where synovial infections occur, disruption of synovial hemeostasis will permanently damage the cartilage as well as prevent the joint from fully healing (Mulon et al., 2016). Infectious arthritis in cattle is a disease that is mostly diagnosed in young animals (Rohde et al., 2000). In calves, more than one joint is usually affected due to haematogenous spread of bacteria from a distant point of infection (Desrochers, 2004a). Septic arthritis is usually characterised by symptoms of severe lameness, joint swelling and pain (Cakir et al., 2019) and is usually associated with failure of passive transfer (FPT) or infection of umbilical remains (Ismail et al., 2007). Infection can cause joint deformities in progressive cases (Desrochers, 2004a).

The physical properties of synovial fluid in calves with arthritis provide useful diagnostic information (Kofler, 1999; Rohde et al., 2000; Ismail et al, 2007; Gokhan & Ozturk, 2008). In various studies conducted in calves with arthritis, it was reported that the viscosity of synovial fluid decreased (Arıcan et al., 1998; Sarıerler, 1999; Gokhan & Ozturk, 2008) and the colour of synovial fluid varied between light yellow-yellow (Van, 1972), yellow-grey (Arıcan et al., 1998), red-pink, dark yellow-brown (Sarıerler, 1999). In the present study, it was observed that the viscosity decreased in all synovial fluid samples and the colour changed from white to pink-red. The isolation of the agent was mostly performed from pink-red coloured synovial fluid samples and this result is similar to the study made by Gokhan & Ozturk (2008).

Joint diseases are an important problem in cattle breeding as they cause loss of productivity in cattle. Many cases had un-

favourable responses to antibiotic treatment. However, early diagnosis is critical for the success of treatment and the return of normal function of the joint (Tsuka et al., 2020). Via examination of synovial fluid, various microorganisms are isolated from arthritic joints. *Staphylococcus* spp., *E. coli*, *Erysipelothrix*, *Trueperella*, *Salmonella,* and (less commonly) *Mycoplasma* spp. are among the most frequently isolated microorganisms (Desrochers, 2004b; Maunsell et al., 2011; Sababoglu et al., 2018). In this study, *T. pyogenes* from 4.61% and *S. aureus*, *M. bovis*, *E. rhusiopathiae* and *E. coli* from 1.54% of synovial fluid samples were isolated. In A retrospective study on bacterial culture of 172 cases of septic arthritis was seen that *T. pyogenes* was the most common bacteria isolated (35% of positive culture in young animal) (Francoz et al., 2002). Similarly, *T. pyogenes* was the dominant species isolated in the present study. However, according to various studies the isolation rates obtained are quite low (Watkins & Sharp, 1998; Sababoglu et al., 2018). It is thought that the isolation method used in these low isolation rates, the number of samples studied may be effective or the animals may have been previously treated with a drug treatment. Also sometimes bacteria may be temporarily absent from the synovial fluid, in which case they may not be isolated by culture (Rohde et al., 2000).

Bacterial culture is one of the most important clinical tools that can be used in the diagnosis of infectious arthritis, in addition to clinical symptoms, radiographic examination, and cytological analysis of synovial fluid (Francoz et al., 2015). However, its role is limited due to the low recovery rates of microorganisms. When combined with the results of cytological analysis of synovial fluid and clinical and radiological examination findings, a positive bacterial culture confirmed the diagnosis of infectious arthritis. However, a negative culture does not change this possibility. This limitation of the usefulness of routine synovial fluid culture is due to the localization of bacteria in the synovial membrane, previous antibacterial applications, and the inherited antibacterial properties of the synovial fluid (Carter, 1991) and the presence of a chronic or non-infectious arthritis should also be taken into account in cases where isolation cannot be performed (Riley & Farrow, 1998; Watkins & Sharp, 1998). In addition, it has been reported that factors such as the presence of some other agents (including viral agents), immunity and the activity of leukocytes, and sampling and laboratory methods affect isolation rates (Goodarzi et al., 2015). In the current study, reasons like those mentioned above may have caused the failure of isolation in 90.77% of the samples.

However, in the current study, the result of direct PCR from synovial fluid samples determined the DNA of *T. pyogenes* in 18 samples (27.7%), the in one sample, DNA of *S. aureus* in 8 samples (12.3%), In three (4.62%) samples, *T. pyogenes* and *S. aureus* were determined together. Also isolated other bacteria (*E. rhusiopathiae M. bovis*) were identified as molecular. In this study isolation rates were lower than PCR findings. This situation made thought that the calves may have been given a drug treatment before as recommended by Herrel et al. (1944) and Balboni et. al. (1945). And also in acute or subacute cases, that insufficient time may not have elapsed for the infecting microorganisms to multiply in sufficient numbers to allow isolation in bacteriological cultures (Kallo et al., 1997).

In the present study, higher prevalence of arthritis was recorded in females (24.62%) than males (23.07%). This result was similar to studies of Rohde et al. (2000) and Ramathan (2007). In contrast, higher incidence in males than females was documented by Dogan et al. (2016) and Yurdakul (2018).

Antibiotic susceptibility test was performed on the isolates obtained in the present study. As a result of the test, the most frequently isolated agent *T. pyogenes* was found to be sensitive to P, TMP-SXT, CN, FFC, AMP and AMC. The results obtained are in parallel with the results of the study conducted by Jost & Sickenger (2021) in calves with arthritis. *E. rhusiopathiae* which is one of the important factors encountered in arthritis, was found to be sensitive to P, E and CIP antibiotics as a result of the antibiogram, similar to the study conducted by Balootaki et al. (2017). All isolates except *M. bovis* were susceptible to amoxicillin and ampicillin. Of the isolates, 14.3% (*M. bovis*) were susceptible to oxytetracycline and 85.7% *(E. coli, S. aureus, T. pyogenes, E. rhusiopathiae*) were resistant. For neomycin, susceptibility rate was 85.7% and resistance rate was 14.3%. *E. coli* and *S. aureus* were susceptible to penicillin, while other isolates were resistant. Also all isolates were sensitive against to danofloxacin.

In addition, MDR positivity was determined in isolates. This situation was similar to study of Motta et al. (2017). Inappropriate or empirical use of antimicrobial agents increases the selection rate of multidrug-resistant bacteria, which is an emerging global threat (Giguère et al., 2010). Based on these findings, the choice of first-choice antimicrobial therapy should be based on the regional in vitro resistance profile (Ribeiro et al., 2015).

CONCLUSION

This study aimed to determine the etiological agents of infectious arthritis, which is one of the causes of lameness in animals, and to investigate the antibiotics that will be used in their treatment in Kars province, where animal husbandry is an important source of economic income. The obtained findings revealed that various microorganisms are the cause of infectious arthritis. This is an important study in terms of determining the antibiotics that can be used in cases of arthritis, which is one of the serious problems of animal husbandry and is an important source of income for local farmers. Of course, it should be kept in mind that the results obtained from this study are in laboratory conditions, and the ability to penetrate the synovial fluid, the amount of drug activity, the pH of the synovial fluid, and the type of pathogen affect the outcome of antibiotic therapy.

DECLARATIONS

Ethics Approval

In the study, the permission associated with taking synovial fluid from 0-3 month-old calves was provided by the local ethics committee with the code of KAUHADYEK/2017-101.

Conflict of Interest

The authors declare that they have no competitive interests

Consent of Publication

No applicable

Author Contributions

Idea, concept, and design: EÇ, İÖ, ÖÇ, CŞE

Data collection and analysis: CŞE, EÇ, AGS, MRC

Drafting of the manuscript: EÇ, AGS, CŞE, ÖÇ, MRC

Critical review: İÖ, ÖA, ÖÇ, AGS, CŞE, EÇ, MRC

Data Availability

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request (E. ÇELİK).

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Research Article

The effect of fermented natural lactic acid bacteria liquid and water-soluble carbohydrate admixture on alfalfa (*Medicago sativa* L.) silage fermentation quality, in vitro digestibility and methane production

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ABSTRACT

This study was carried out to determine the effect of fermented natural lactic acid bacteria liquid and water-soluble carbohydrate (WSC) admixtures on fermentation quality, *in vitro* digestibility, and methane production of alfalfa silage. In the study, analyses (pH, total lactic acid bacteria (LAB), yeast, mold, lactic acid (LA), acetic acid (AA), and LA/AA) of naturally fermented lactic acid bacteria liquid (PFJ) prepared with 3% fructose addition to alfalfa plants were conducted. Treatments included alfalfa (control, C), alfalfa + PFJ (PFJ-C), alfalfa + PFJ + 1.5% molasses (PFJ-CM), alfalfa + PFJ + 1.5% fructose (PFJ-CF), and alfalfa + PFJ + 1.5% sucrose (PFJ-CS). In the study, the differences between the groups were insignificant in the crude ash (CA) and neutral detergent fiber (NDF) values of the silage. On the other hand, the differences between the groups were statistically significant in dry matter (DM), crude protein (CP), acid detergent fiber (ADF), *in vitro* organic matter digestion (IVOMD), metabolizable energy (ME), and methane (CH_4) values. Compared to the control group (57.45%), increases in IVOMD and ME were observed in silage obtained by adding PFJ (58.67%), molasses (59.35%), fructose (63.83%), and sucrose (62.96%). When the fermentation characteristics (pH, ammonia nitrogen (NH_3-N), lactic acid (LA), acetic acid (AA), yeast-mold, and carbon dioxide (CO_2) after aerobic stability) of the silage were analyzed, the differences between the groups were statistically significant. When the CO_2 content and post-aerobic yeast mold values (PAYMV) of the silage were examined, it was observed that there was a decrease in all experimental groups compared to the control group. The LA and AA values of silage increased in all experimental groups compared to the control group. Groups with PFJ had a positive effect on nutritional values, digestibility properties, and fermentation properties compared to the control group. However, considering the groups with PFJ, it can be said that the addition of PFJ-CF is better for silage quality.

INTRODUCTION

Roughage needs to be provided on a regular basis all year round in order for livestock production to be affordable and sustainable, particularly in terms of ruminant nutrition. High-quality, abundant, and cheap roughage sources provide economic profit to the enterprise by minimizing the use of expensive concentrate feeds in animal feeding (Jelan, 2011). Alfalfa plant, which is a source of roughage, is a perennial and polymorphous legume forage plant and is rich in nutrients, especially protein. Since the leaf stalks are thin and weak, significant nutrient losses occur during the period from drying to feeding to animals (Tıknazoğlu, 2009). In regions with high rainfall, nutrient losses occur due to microbial degradation due to incomplete drying. In order to prevent these physical and microbiological losses, ensiling alfalfa plants can be a reasonable solution. Since alfalfa is deficient in water-soluble carbohydrates (WSC) and has a high buffer capacity, additives are necessary during ensiling (Xie et al., 2021). For this purpose, various WSC sources (Gao et al., 2021), and bacterial inoculants (Sun et al., 2021) can be added to the silage to ensure the desired fermentation in alfalfa silage. Lactic acid bacteria (LAB) used in silage fermentation play an essential role in improving silage quality and increasing silage shelf life (Okoye et al., 2023). Lactic acid bacteria are a group of microorganisms that provide the desired fermentation as they cause almost no loss of nutrients during the fermentation process of silage. The main function of LAB in silage is to ferment WSC into organic substances as end products (da Silva et al., 2017). The production of organic acids by LAB depends on the amount of WSC present in the fresh crop and the nature of the bacterial strains used (Kim et al., 2021). The amount and variety of simple sugars in the feed plant, the microbial community in the fresh crop, the LAB strains' resistance to low pH and ability to use particular substrates, and the plant material's capacity, including buffering, all affect how competitive lactic acid bacteria are with other microorganisms in the silo environment (Karademir and Karademir, 2003). Due to the abundance of epiphytic LAB bacterial species, their synergistic action, ease of practical and cost-effective preparation, and numerous advantages, including the ability to use fermented lactic acid liquid (PFJ) as an alternative to commercial LAB inoculants, PFJ has become increasingly popular in recent years (Sun et al., 2021). In this study, it was aimed to evaluate the effect of a PFJ mixture with different WSC sources on the chemical properties and digestibility of alfalfa silage, to determine which WSC source works more effectively with PFJ, and to find out which one is more appropriate to use in future studies.

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MATERIALS and METHODS

Study design and silage preparation

Using the technique described by Masuko et al. (2002), fermented natural LAB liquid was created in this work by adding fructose to the alfalfa plant. The 1000 ml of distilled water and 1000 g of fresh alfalfa plant were blended for two minutes to shred the mixture. The obtained plant liquid mixtures were filtered using two layers of cheesecloth, and 3% fructose was added to the plant liquid mixtures (PFJ), which were then placed in bottles and incubated at 30°C for 72 hours. The alfalfa plant was used as silage raw material in the study. Fresh alfalfa was harvested and wilted for 24 hours during the early flowering period (terrestrial climate, 37.8°N, 38.46°E, 518 m altitude) in Şanlıurfa province. The wilted material (DM 266 g/kg FW) was chopped into 1-2 cm lengths. In ensiling, 1 ml of fermented natural LAB liquid was added to 1 kg of alfalfa plants. 40 ml/kg of distilled water was added to guarantee homogeneity in all the silage groups created for the study. According to the Tempo automatic bacteria counter test method, the total LAB count in the fresh silage material was calculated using the procedure described by Güney and Ertürk (2020), and it was repeated three times for each group. The buffering capacity of the fresh alfalfa used in the study was determined according to the method reported by Playne and McDonald (1966). In the study, experimental groups were formed from non-additive alfalfa (control, C), alfalfa with added PFJ (PFJ-C), alfalfa with added PFJ and 1.5% molasses (PFJ-CM), alfalfa with added PFJ and 1.5% fructose (PFJ-CF), and alfalfa with added PFJ and 1.5% sucrose (PFJ-CS). With four repetitions, each silage group was compressed into 1.5-liter glass jars, which were then tightly sealed. Silages were stored at room temperature for 60 days in a dark environment.

Fermentation profile analysis

After the silage were opened, the silage subsamples (25 g) were thoroughly shaken with 100 ml of sterile deionized water to measure the pH using a glass electrode and pH meter (Hanna Instruments, Analog pH/ORP meter, Romania) (Polan et al., 1998), and then they were filtered using qualitative filter paper before being stored at -20°C for additional ammonia-nitrogen (NH_3-N) and organic acid analyses. The silage samples underwent NH_3-N analysis using the procedure described by Broderick and Kang (1980), and using a high-pressure liquid chromatography device (HPLC) (Shimadzu SPD M20A Detector (DAD), Shimadzu LC-20 AD HPLC pump, Shimadzu cto-20ac Colum oven, Shimadzu SIL-20 ADHT Autosampler, Icsep Coregel (87H3 colon)), the organic acid contents (known as lactic, butyric, acetic, and propionic acid) were determined in accordance with the technique described by Suzuki and Lund (1980). The silages obtained in the study were subjected to an aerobic stability test (determination of CO_2 production values) for five days (Ashbell et al., 1991).

While the acid detergent insoluble fiber and neutral detergent insoluble fiber analyses of the silage obtained from the alfalfa plants used as silage material in the study were carried out in accordance with Van Soest et al. (1991), the raw nutrient content, such as dry matter, crude ash, and crude protein analyses, were carried out in accordance with AOAC (2005). After the silage ingredients and the produced silages were dried at room temperature and ground in a laboratory mill to pass through a 1 mm sieve, the raw nutrient analyses were conducted. The IVOMD of the silages and the metabolizable energy (ME) and methane (CH_4) content of the forages obtained in the study were determined according to the method reported by Menke et al. (1988). The method described by Filya et al. (2000) was used in the study to determine the yeast and mold content of silage groups.

Statistical method

In the study, One-Way Analysis of Variance (One-Way Anova) was used to determine whether the data obtained from the groups were widely different. Duncan's multiple comparison tests were used to control the significance of the difference between the groups, and $p<0.05$ was considered significant. For this purpose, the IBM SPSS 20 (1991) software program was used.

RESULTS

In the present study, the LAB count, yeast, mold, lactic acid, acetic acid, LA/AA ratio, and pH values of fermented natural LAB liquid (PFJ) obtained from alfalfa plants with 3% fructose supplementation were determined as 3.2×10^{11} cfu/ml, 3.8×10^5 cfu/ml, 2.6×10^5 cfu/ml, 160.38 g/kg DM, 38.12 g/kg DM, 4.2, 3.60, respectively, as indicated in Table 1. The LA/AA ratio of 4.2 in the PFJ employed in the present study reveals that homolactic activity is more intense in fermentation, according to Zhang et al. (2010).

Table 1. Analysis values of naturally fermented lactic acid bacteria liquid prepared from alfalfa plant by adding 3% fructose.

	LAB	LA	AA	LA/AA	pH	Yeast (cfu/ml)	Mold (cfu/ml)
PFJ	$3.2*10^{11}$	160.38	38.12	4.2	3.60	$3.8*10^5$	$2.6*10^5$

LAB: Lactic acid bacteria cfu/ml, LA: Lactic acid g/kg DM, AA: Acetic acid g/kg DM

Table 2. Analysis of fresh alfalfa plant used as silage raw material.

	LAB Log10	Yeast Log10	Mold Log10	BC
Fresh alfalfa	4.38	5.66	4.04	660

LAB: Lactic acid bacteria, BC: Buffering capacity (meq/kg DM)

The analysis of the fresh alfalfa plant used as silage raw material in the study is presented in Table 2.

The nutrient contents, IVOMD, ME, and *in vitro* CH_4 values of the silage groups are provided in Table 3. According to Table 3, while the differences between the groups were found to be statistically insignificant ($p>0.05$) in the CA and NDF values of the silage, the differences between the groups were found to be statistically significant ($p<0.05$) in the DM, CP, ADF, IVOMD, ME, and CH_4 values.

Table 3. Nutrient contents, IVOMD, ME and CH_4 values of silages

	DM	CA	CP	ADF	NDF	IVOMD	ME	CH_4
Control	29.47^{c}	10.63	20.02^{b}	32.41^{ab}	40.78	57.45^{b}	8.50^{c}	14.05^{ab}
PFJ-C	30.57^{b}	10.69	20.38^{ab}	33.83^{a}	42.52	58.67^{b}	8.69^{bc}	13.86^{ab}
PFJ-CM	30.95^{b}	11.23	20.41^{ab}	32.92^{a}	42.77	59.35^{b}	8.77^{bc}	14.55^{a}
PFJ-CF	31.98^{a}	10.96	20.24^{b}	30.93^{b}	42.37	63.83^{a}	9.46^{a}	12.67^{c}
PFJ-CS	31.24^{b}	10.87	20.74^{a}	33.19^{a}	41.42	62.96^{a}	9.09^{ab}	13.49^{bc}
SEM	0.212	0.101	0.080	0.307	0.290	0.668	0.097	0.190
P	0.000	0.366	0.047	0.017	0.138	0.000	0.003	0.009

$^{a-c}$: Values with different letters in the same column were found to be different ($P<0.05$); DM: Dry matter, %; CA: Crude ash DM%; CP: Crude protein, DM%; ADF: Acid detergent insoluble fiber, %DM; NDF: Neutral detergent insoluble fiber, %DM; IVOMD: *In vitro* organic matter digestion, ME: Metabolizable energy, CH_4: *In Vitro* methane gas (%), SEM: Standart Error Mean

The fermentation characteristics of the silage groups are presented in Table 4. When the fermentation characteristics (pH, NH_3-N, LA, AA yeast mold after aerobic stability) and CO_2 of the silage prepared in the study were analyzed, the differences between the groups were determined to be statistically significant ($p<0.05$).

Table 4. Fermentation characteristics of silages

	pH	NH_3-N/TN	PAYMV	LA	AA	CO_2
Control	5.09^{a}	11.95^{a}	2.48^{a}	12.07^{e}	7.62^{e}	5.81^{a}
PFJ-C	4.83^{b}	9.81^{ab}	1.12^{b}	13.48^{d}	9.68^{d}	2.24^{b}
PFJ-CM	4.67^{c}	8.05^{bc}	0.00^{c}	16.34^{c}	20.16^{a}	1.52^{c}
PFJ-CF	4.31^{d}	7.02^{c}	0.00^{c}	23.35^{a}	12.26^{c}	1.22^{d}
PFJ-CS	4.34^{d}	6.40^{c}	0.00^{c}	17.08^{b}	14.37^{b}	1.04^{e}
SEM	0.070	0.546	0.261	1.042	1.155	0.473
P	0.000	0.000	0.000	0.000	0.000	0.000

$^{a-c}$: Values with different letters in the same column were found to be different ($P<0.05$); NH_3-N/TN: Ammonia nitrogen, CO_2: Carbondioxide g/kg DM, LA: Lactic acid g/kg DM, AA: Acetic acid g/kg DM, PAYMV: Post aerobic yeast-mold values.

DISCUSSION

Total lactic acid bacteria (LAB) values (3.2×10^{11} cfu/ml) of PFJ prepared in this study were higher than the values obtained from the study of Aydın and Denek (2019) and similar to the values obtained from the study of Aydın and Denek (2022). The yeast and mold values in the natural LAB liquids used in the study had higher yeast and mold values (5.78 cfu/ml and 5.41 cfu/ml, respectively) than the fermented liquid made from the alfalfa plant by Tao et al. (2017), which had values of 5.34 cfu/ml and 4.18 cfu/ml, respectively. The pH value of PFJ prepared from alfalfa plants was calculated at 3.60. The LA and AA values in PFJ were 160.38 and 38.12 g/kg DM, respectively. The LA (154.2 g/kg DM) and AA (36 g/kg DM) values found in the PFJ prepared by Bureenok et al. (2005) agree with the present study. In the studies conducted so far, molasses, sucrose, and glucose additives were used as nutrient sources in the preparation of fermented lactic acid bacteria liquid, while no study with fructose addition was encountered. The possible reason for the microbiological and chemical differences between the fermented natural lactic acid bacteria liquids may be due to the type of plants used in the studies, the source and amount of WSC used, and the incubation time.

When the data was analyzed, the LAB count of the fresh alfalfa plant was determined as Log10 4.38 cfu/g. This value was higher than the value reported by Sun and Yang (2021), lower than the value reported by Silva et al. (2020), and Wang et al. (2023), and similar to the value reported by Si et al. (2023). A meta-analysis by Oliveira et al. (2017) reported that LAB counts above 10^5 cfu/g could ensure efficient fermentation, while values below 10^4 cfu/g might decrease DM recovery and

increase ammonia nitrogen concentration. In the study, the buffering capacity of the alfalfa plant was found to be 660 meq/kg DM. This value was lower than the values reported by Turan and Önenç (2018) (720 meq/kg DM), and higher than Sun et al. (2021) (583, 425). In our study, the total yeast count of the alfalfa plant was determined as Log10 5.66 cfu/g and the mold count as Log10 4.04 cfu/g. These yeast values are higher than the values reported by Wang et al. (2023) and Si et al. (2023) in their studies, while the mold value of Wang et al. (2023) is lower than our study. The yeast and mold count of Silva et al. (2020) in alfalfa plants are similar to our study. The number and types of natural microorganisms in plants vary according to environmental conditions, location of the silo, time (season), degree of contamination, plant species, plant variety, and DM content (Kılıç, 1986).

When the DM contents of the silages obtained by adding WSC such as molasses, fructose, and sucrose at a rate of 1.5% in addition to PFJs were examined compared to the control group, increases in DM values were observed in all groups, while the highest value (PFJ+CF) was detected.

When the ADF values of the silages were analyzed, the (PFJ+CF) addition group was found to be lower than the other silage groups. The decrease in ADF value is similar to Wang et al. (2023) and Si et al. (2023). It was found to be insignificant in terms of NDF values. The report that LAB had little or no degrading effect on the cellulose value was consistent with our study (Muck, 1996).

In the study, when the IVOMD and ME values of the silages obtained by combining WSC such as molasses, fructose, and sucrose at a rate of 1.5% in addition to PFJs were examined, increases were observed in the PFJ+CF and PFJ+CS groups compared to the control group. This increase supports the report of Okuyucu (2018) that LA is the main fermentation product in silages, and LA is fermented in the rumen and evaluated by ruminants, accordingly, increasing the IVOMD and ME values.

When the percentage CH_4 values of silages were analyzed, the lowest value was observed in the PFJ+CF group. Carbohydrate sources are converted into volatile fatty acids (VFA), H_2, and CO_2 in the rumen. H_2 and CO_2 released as a result of rumen fermentation cause methane (CH_4) gas formation (methanogenesis) by methanogenic microorganisms (bacteria, archaea, and protozoa) (Hegarty and Klieve, 1999). It is stated that when bacteria utilizing lactic acid convert this lactic acid to propionic acid, the production of hydrogen and formic acid, which are precursors of methane, decreases, resulting in a decrease in methane production (Sarıpınar and Sulu, 2005). The PFJ+CF-supplemented group had the highest level of lactic acid, and it is believed that methane emission is inhibited by bacteriocins produced by lactic acid bacteria species that use fructose in silage more effectively (Hegarty and Klieve, 1999).

The pH values obtained from all supplemented silage groups were lower than the control group values. The highest pH value (5.09) was obtained from the control group silage, and the lowest pH values 4.31 and 4.34 were determined in the PFJ+CF and PFJ+CS groups, respectively. It is expected that the addition of WSC to alfalfa plants will decrease the pH value of silage. The pH values in the supplemented groups in our study are in accordance with the report of Kung and Shaver (2001) that the pH value should be in the range of 4.3–4.7 for quality legume silages.

Ni et al. (2017) reported that adding sucrose to silages boosted the growth of Lactobacillus and Pseudomonas while preventing the formation of unwanted Enterobacter. In addition, Zi et al. (2022) reported that the addition of WSC altered the bacterial assemblage in silage, increasing the number of acids producing *Megamonas, Bacteroides, Megasphaera, Faecalibacterium, Stenotrophomonas,* and *Bifidobacteriums* and decreasing the number of *Weissella* and *Enterobacteriums*. The strongest of the acids formed during silage fermentation is lactic acid, and with the addition of an effective LAB inoculant, a large volume of lactic acid production and subsequently a low pH value are obtained in silage (Muck, 1996). When the pH and LA values of silage were evaluated together, a negative relationship was observed. This relationship was consistent with our data in the PFJ+CF group, where lactic acid content was 23.45 g/kg, DM was the highest, and pH 4.31 was the lowest.

Silage groups prepared with the addition of molasses, fructose, and sucrose showed a decrease in NH_3-N values when compared to the control group in the study. Gao et al. (2021) reported that the addition of molasses and fructose to alfalfa silage reduces the nitrogen content of ammonia. With the use of inoculants and WSC, the pH of the silage decreased quickly. This was likely caused by the inhibition of *Enterobacter, Clostridium*, and other microorganisms that consume the crude protein and inhibit the plant's protease activity, preserving some true proteins and lowering the concentration of NH_3-N in the silage (Muck, 1996).

Interpretation of the results of the LA and AA values of silages shows that there were increases in all experimental groups compared to the control group. Table 3 indicates that the highest lactic acid content and the lowest pH value were observed in the PFJ+CF group. Gao et al. (2021) reported an increase in LA values due to the addition of molasses and fructose to alfalfa silage, which is in agreement with the present study. In the groups with WSC additives, the highest AA value was observed in the molasses-added group, while the lowest AA value was observed in the fructose-added group. This could be because molasses contains nitrogenous chemicals that microbes can use in addition to sucrose (Otero et al., 1993).

The silage groups to which all additives were added showed higher levels of acetic acid content and lower levels of CO_2 and yeast mold values when the data was analyzed in comparison to the silage in the control group. Yeast and mold contamination reduces silage quality (Blajman and Vinderola, 2020). It is recognized that yeasts in the silage environment during the aerobic period produce CO_2 intensively. In this study, the CO_2 values of silages prepared by adding various additives were found to be low. The amount of acetic acid produced by heterolactic LAB fermentation in the silages in the additive groups supports the report that it has an inhibitory effect against microorganisms that lead to the deterioration of silage, prevents the growth and activity of yeasts, and reduces

CO_2 production, i.e., improves aerobic stability values (Ali et al., 2020).

Lactic acid bacteria are characterized by degrading different carbohydrates at different levels. The higher the molecular weight of the carbohydrate type, the lower the level of fermentation. More complex carbohydrates, such as sucrose and polysaccharides, are more difficult to break down than monosaccharides. In addition, microbial and plant enzymes play an important role in this breakdown process (Kılıç, 1986). When all parameters were analyzed, the higher silage quality in the fructose-supplemented groups compared to the other groups can be attributed to the better utilization of fructose, which is a monosaccharide, by LAB species in the PFJ and in the silage.

CONCLUSION

The purpose of this research was to ascertain how alfalfa silage fermentation quality, *in vitro* digestibility (IVOMD), and methane generation were affected by the addition of WSC and fermented natural lactic acid bacteria. The pH, CO_2, yeast, and mold values of silages decreased in all experimental groups compared to the control group silage. By preventing the development and activity of yeasts and molds as well as the microorganisms responsible for silage spoiling, the quantity of acetic acid generated by heterolactic LAB fermentation in the silages of the addition groups increased the aerobic stability values. The amount and composition of pH, NH_3-N, and organic acids (acetic acid, butyric acid, and lactic acid) formed during silage fermentation determine the quality of fermentation. Especially silage groups with low pH and NH_3-N can be considered well-fermented silages. In terms of all parameters, it was concluded that the addition of 1.5% fructose to PFJs prepared by adding 3% fructose had positive effects on silage fermentation, *in vitro* organic matter digestion, metabolizable energy (ME), and *in vitro* methane gas formation.

DECLARATIONS

Ethics Approval

Harran University Local Ethics Committee, the letter no: 2022/006/06

This study is not subject to HADYEK permission in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees".

Conflict of Interest

There is no conflict of interest between the authors.

Consent for Publication

The authors give permission for publication..

Author contribution

Idea, concept and design: S.S.A, N.D., M.A.

Data collection and analysis: N.K., Ş.T.

Drafting of the manuscript: S.S.A, N.D., M.A., N.K., Ş.T.

Critical review: S.S.A, N.D., M.A., N.K., Ş.T.

Data Availability

The data used to prepare this manuscript are available from the corresponding author when requested.

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Research Article

Morphological investigation of arcus aortae and branches in badger (*Meles meles*)

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ABSTRACT

The branches originating from the arcus aortae formed by the aortae after exiting the heart differs between animals. In this study, it was aimed to examine the morphology of the arcus aortae in the badger (*Meles meles*). Two adult badgers, which died as a result of a traffic accident at different times and were brought to the anatomy laboratory. Thorax was dissected and the heart and aortae were exposed. It was observed the heart was located between 3rd-7th costae and was connected to the diaphragma with the ligamentum sternophericardiacum. It was determined that truncus brachiocephalicus and arteria subclavia sinistra was originated from the arcus aortae at the level of 4th costae. It was determined that the first branch of truncus brachiocephalicus was arteria carotis communis sinistra and then divided into arteria subclavia dextra and arteria carotis communis dextra, respectively. It was seen that arteria vertebralis, truncus costocervicalis, arteria cervicalis superficialis and arteria thoracica interna originated from arteria subclavia sinistra. Branches of arteria subclavia dextra were found to be similar to arteria subclavia sinistra. It was determined that the branches originating from the arcus aortae were similar to those in cats and dogs. It is thought that this study will contribute to the anatomical knowledge of the endangered badger, which is under protection.

INTRODUCTION

Badger (*Meles meles*) belongs to the Mustelidae subfamily and order Carnivora (Demirsoy, 2003). The badger is widespread in the world, and spreads coast to 2000 m, from Trakya to Eastern Anatolia in Turkey (Ozen and Ulucay, 2010). The badger is hunted for its valuable skin and some of its by-products (Sapundzhiev et al., 2019). In Turkey, the badger population is endangered and therefore it has been taken under protection (Pamukoglu ve Demir, 2001).

There are various studies about the badger's biology, ecology and habitat (Ozen and Ulucay, 2010; Pamukoglu and Demir, 2001; Roper, 2009; Pamukoglu and Tuncer, 2014) as well as several studies on its anatomy. Anatomic studies were on badger gastrointestinal anatomy (Stark et al., 1987), pancreatic morphology (Ozdemir, 2005), skeleton macro-anatomy (Atalar and Cakır, 2004; Spataru, 2016), ear ossicles morphology and morphometry (Martonos et al., 2022), tongue morphology (Haligur et al., 2022). In the literature research, no study has been found about the aortae, which is the beginning of the circulatory system.

The curvature of the aortae after leaving the left ventricle of the heart is called arcus aortae. (Getty, 1975; Dursun, 2006). The head, neck, forelimbs, some organs in the thoracic cavity, and the anterior part of the chest are supplied by two large vessels, the arteria subclavia and arteria carotis communis, which originate from the arcus aortae. (Evans et al., 1993). In Equidea and ruminantia these two arteries stems from the truncus brachiocephalicus which originates from the arcus aortae. In carnivores and sus, the arteria subclavia sinistra originates directly from the arcus aortae, not from the truncus brachiocephalicus (Chiasson and Booth, 1982; Dyce et al., 2002).

In this study, it was aimed to morphologically examine the arcus aortae and the branches originated from the arcus aortae in the badger.

MATERIALS and METHODS

In this study, two adult female (9.21-11.72 kg) badgers (*Meles meles*), which died in a traffic accident at different times around the Ceyhan district of Adana province were used. They were brought to the Ceyhan Veterinary Faculty Anatomy Department and stored in deep freezer until study. Thoracic cavity of the badger was opened with the classical dissection method, and the tissues around the heart and aortae were removed with the same method. Tissues were fixed in 10% formaldehyde solution. The dissected materials were photographed with a digital camera. Nomina Anatomica Veterinaria (2017) was used as a reference in anatomical terminology.

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RESULTS

In this study it was determined that the heart of badger lies in a rather oblique manner in the thoracic cavity, at the mediastinum, and between the level of the third to the level of seventh costae.

It was observed that the arcus aortae (Ar) started after the aortae ascendens at the level of the fourth to the level of fifth spatium intercostale and continued up to the level of the sixth vertebrae thoracalis (Figure 1). The arcus aortae showed a craniodorsal curvatura. It was seen that in both of the badgers, firstly the truncus brachiocephalicus (Tr) (Figure 2) and then the arteria subclavia sinistra (Ss) (Figure 2) originated from the arcus aortae at the level of fourth costae. It was indicated that arteria carotis communis sinistra (Cs) (Figure 2), arteria carotis communis dextra (Cd), and arteria subclavia dextra (Sd) (Figure 2) originated from the truncus brachiocephalicus (Tr), respectively at the level of the first to the level of second costae near the apertura thoracis cranialis. The formation of truncus bicaroticus could not detected in the both of the badgers.

It was determined that firstly the arteria vertebralis (Av) originate from arteria subclavia sinistra (Ss) at the medial level of first costae, and then trunchus costocervicalis (Tc) originate at the level of the apertura thoracis cranialis (Figure 2). In addition, it was observed that arteria cervicalis superficialis and arteria thoracica interna originated from arteria subclavia sinistra after the origin of the truncus costacervicalis. Branches of arteria subclavia dextra were found to be similar to arteria subclavia sinistra.

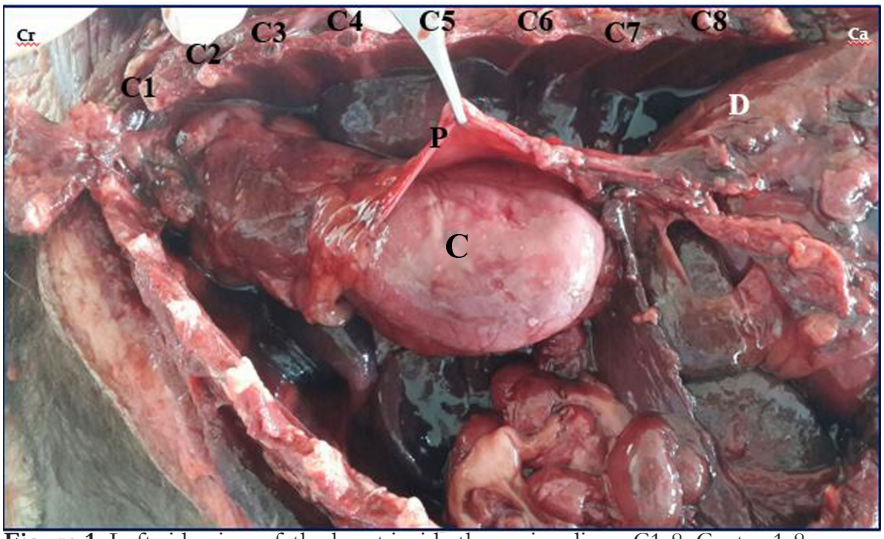

Figure 1. Left side view of the heart inside the pericardium. C1-8: Costae 1-8, Ca: Caudal, Cr: Cranial, D: Diapragma, C: Cor, P: Pericardium

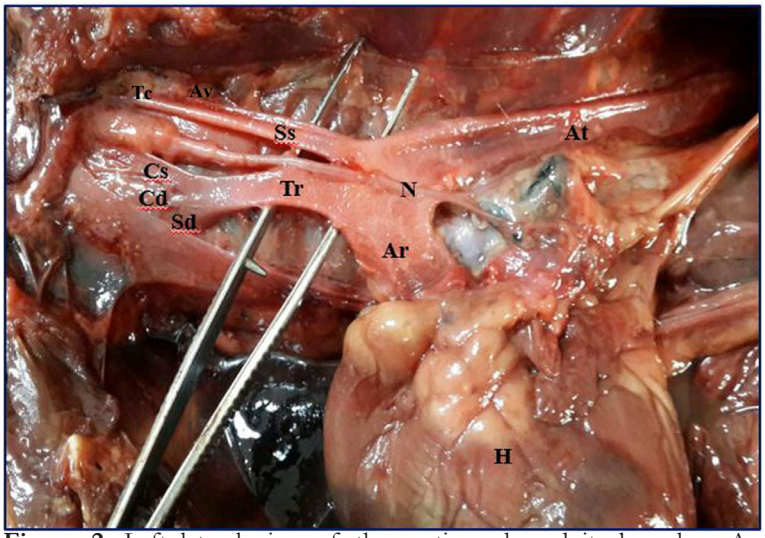

Figure 2. Left lateral view of the aortic arch and its branches. Ar: Arcus aortae, At: Aortae thoracica, Cd: Arteria carotis communis dextra, Cs: Arteria carotis communis sinistra, H: Cor, N: Nervus vagus Sd: Arteria subclavia dextra, Ss: Arteria subclavia sinistra, Tr: Truncus brachiocephalicus, Tc: Trunchus costocervicalis, Av: arteria vertebralis

DISCUSSION

The heart is located on the left midline in the mediastinum, and lies between the level of third to the level of fifth costae in the ruminant and sus, the level of third to level of sixth costae in the equide and level of third to the level of seventh costae in cats and dogs (Dursun, 2006; Chiasson and Booth, 1982). 2013). The location of the badger heart is similar to the cat and dog findings in the literature (Getty, 1975; Dyce et al., 2002).

It has been reported that the arcus aortae begins at the level of the second spatium intercostale in the rabbit (Ekim and Dursun, 2011). In the current study, it was determined that the arcus aortae in the badger started at the level of the fourth to the level of fifth spatium intercostale. In addition, it was observed that the arcus aortae in the badger continued up to the level of the sixth vertebrae thoracalis, as in the rabbit (Ekim and Dursun, 2011).

It has been indicated that truncus brachiocephalicus originates from arcus aortae as a single branch in equidea (Getty, 1975; Dyce et al., 2002; Budras et al., 2009) and ruminantia (Getty, 1975; Dursun, 2006; Dyce et al., 2002). But in cats, dogs (Getty, 1975; Dursun, 2006; Chiasson and Booth; 1982; Dyce et al., 2002) and squirrels (Sadeghinezhad et al., 2015), first the truncus brachiocephalicus and then the arteria subclavia sinistra are originated from the arcus aortae. It was reported that the truncus brachiocephalicus and arteria subclavia sinistra were originated from the arcus aortae at the level of the second costae in chinchilla (Ozdemir et al., 2008), at the level of the fifth spatium intercostale in fox (Karakulum and Ozcan, 2013). In addition to these two main arteria, arteria anonyma was also originated from arcus aortae in rat (Teke, 2000). It was reported that in spiny mice (Oto et al., 2010) and porcupines (Atalar, 2011), truncus brachiocephalicus, arteria carotis communis sinistra and arteria subclavia sinistra originate from arcus aortae, whereas in rabbit (McLaughlin and Chiasson, 1979) the arteria anonymus and arteria subclavia sinistra originate from the arcus aortae. In a study conducted in rabbits, it was reported that while the truncus brachiocephalicus firstly originated from the arcus aortae in 9 animals, arteria carotis communis sinistra, arteria carotis dextra and arteria subclavia sinistra were originated from arcus aortae respectively in one animal. In this study, similar findings were obtained in cats, dogs (Getty, 1975; Dursun, 2006; Chiasson and Booth, 1982; Dyce et al., 2002), chinchillas (Özdemir et al., 2008) and squirrels (Sadeghinezhad et al., 2015), but not in rats (Teke, 2000) and rabbits (Ekim and Dursun, 2011; McLaughlin and Chiasson, 1979).

It has been reported that arteria carotis communis sinistra, arteria carotis communis dextra, and arteria subclavia dextra originate from the truncus brachiacephalicus respectively in cats and dogs (Getty, 1975; Dursun, 2006; Chiasson and Booth, 1982; Dyce et al., 2002). As stated in rabbits (Ekim and Dursun, 2011), truncus bicaroticus structure was not found in this study. It has been reported that the sus has truncus bicaroticus and the arteria subclavia dextra immediately originates from the arcus aortae (Getty, 1975). In this study, it was observed that the truncus brachiocephalicus was in the form of a single root and arteria carotis communis sinistra, arteria carotis communis dextra and arteria subclavia dextra was originated from this root.

CONCLUSION

In present study, the morphological structure of the arcus aortae and its branches of badger (*Meles meles*) was revealed. It was determined that the branches originating from the arcus aortae were similar to in cats and dogs. The study was carried out on two animals in order not to reduce badger's population in the wild life. It is thought that this study will contribute to the anatomical knowledge of the endangered badger, which is under protection.

DECLARATIONS

Ethics Approval

This study was carried out of with the permission of the Ministry of Forestry and Water Affairs Nature Conservation and National Parks General Directorate. (numbered 38002405-445.05-177733) and the approval of Çukurova University Animal Experiments Local Ethics Committee numbered 2017/6-6.

Conflict of Interest

The authors declare that there have no conflict of interests.

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: AH, SÖ

Data collection and analysis: AH, SÖ

Drafting of the manuscript: AH

Critical review: SÖ

Data Availability

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

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Research Article

Investigation of the immunomodulatory effect of inactive parapoxvirus (iPPVO) on infectious bovine rhinotracheitis (IBR) vaccine in cattle

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ABSTRACT

In the study, alterations in antibody titers, proinflammatory and antiinflammatory cytokine levels were determined in serum samples collected at various periods before and after administration of inactive IBR and iPPVO to cattle. It was aimed to investigate the immunomodulatory effects of inactivated parapoxvirus ovis (iPPVO) in cattle vaccinated with inactivated-IBR vaccine. In the study, 40 unvaccinated, clinically healthy cattle of different breeds aged older than 3 months were used. Three groups of cattle were formed as control group 1 (n=10), control group 2 (n=10) and experimental group (n=20). iPPVO was applied to the cattle in the control group 1 and experimental groups on the 0, 2nd, and 4th days. Blood samples were collected from all the animals after 6th hours of the injections applied on 0th and 4th days of the study. Commercially available ELISA kits were used to determine serum levels of of IL-2, IL-6, IL-12 and IFN-γ. Furthermore, virus neutralization test was also performed to detect virus neutralizing antibody titres. In the present study, serum levels of IL-2, IL-6 IL-12, and IFN-γ levels were found to be significantly higher in the experimental group compared to that of the control group 1 and control group 2 ($p<0,05$). The differences between control group 1 and control group 2 groups were not statistically significant.

In conclusion, iPPVO increased the levels of cytokines in IBR vaccinated cattle due to its immunomodulatory effects. In addition, virüs neutralizing antibody titers were found to be significantly higher in cattle that received vaccine and iPPVO.

INTRODUCTION

Infectious Bovine Rhinotracheitis (IBR) is caused by Bovine herpes virus-1 (BoHV-1). Bovine herpes virus-1 (BoHV-1) causes fertility disorders, abortions and fatal systemic diseases in newborns by affecting the nervous and genital system, especially the respiratory system in cattle (Jones and Chowdhury, 2010; Raaperi et al., 2012). BoHV-1 infected cattle are latent throughout their lives. The virus may become reactivated due to stress or immunosuppressors (Kook et al., 2015; Winkler et al., 2000). In general, the elimination of stress factors and the use of immunomodulators in vaccination provide better immunization. Inactivated Parapoxvirus ovis D1701 strain (iPPVO) has been licensed as an immunostimulant drug in the veterinary field (Coskun, 2017). It has been declareted that iPPVO reduces susceptibility and has antiviral activity against herpes simplex and hepatitis B infection in mice by activating cytokine (TNF-α, IFN-γ, IL-12 and IL-18) induction against these viruses. In a study in mice, it was determined that iPPVO administration stimulated the synthesis of TNF-α (16th and 24th hours) and IL-6 (12th, 16th and 24th hours) and caused changes in IL-10 and IL-12 levels. It has been reported that the increase in cytokine levels can be attributed to the immunomodulatory activity of iPPVO (Dinarello, 2000). However, in the literature review, it was seen that there was no research on the use of iPPVO in vaccination with inactivated vaccines and its effects on the synthesis of cytokines, which is the first response mediator of the immune system after vaccination (Friebe et al., 2004; Weber et al., 2003).

In this study, it was aimed to investigate the immunomodulatory effect of iPPVO (Zylexis® flk) in cattle vaccinated with inactivated-IBR vaccine by determining the virus neutralizing antibody titers, proinflammatory and antiinflammatory cytokine levels.

MATERIALS and METHODS

Animals

In the study, 40 clinically healthy cattle, older than 3 monthes, various breeds and sexes were used. All the animals used in the study were not vaccinated with IBR vaccine before the study. Cattle were divided into 3 groups as follows; Control group-1 (n=10), Control group-2 (n=10) and Experimental group (n=20). This study was approved by the Local Ethics Committee for Animal Care of the Burdur Mehmet Akif Ersoy University (12/12/2018- decision number: 470). Consent forms were signed by the animal owners.

Administration of iPPVO (Zylexis® flk) and IBR vaccine (Rispoval®)

Contro group 1: A single dose of 2ml of iPPVO (Zylexis® flk) were administered intramuscularly on 0th, 2nd and 4th days of the study. Blood samples were taken at the 6th hour after the each injection.

Control group 2: IBR vaccine (Rispoval®) was administered to the each animal subcutaneously at a dose of 2 ml on the

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4th day of the study. Blood samples were taken at before and the 6th hour of the 4th days.

Experimental group: 20 cattle in the experimental group were ricieved 2 ml iPPVO (Zylexis® flk) intramuscularly on the 0th and 2nd days, and then at the 4th days iPPVO and 2 ml of IBR vaccine (Rispoval®) were given simultaneously. Samples were taken at the 6th hour of the 0th and 4th days of the injections.

Collection of Blood Samples

Samples were collected from all the animals on 0th, 2nd, 4th days of the study into plane tubes. These samples were then used to measure cytokine levels (IL-2 IL-6, IL-12, IFN-γ). Serum samples were also collected on 0th, 7th, 21st days and used to determine virus neutralizing antibody titres. All the serum samples were kept at at -20°C until used. Samples were also collected on 0th and 4th days into tubes with EDTA and used to determine hematological parameters.

ELISA Tests

Commercial Bovine specific ELISA kits were used to determine (ELISA, YL biont, Shanghai, CHINA) IL-2, IL-6, IL-12 and IFN-γ concentrations in the collected serum samples. The ELISA was performed according to the manufacturer's instructions. The optical density (OD) of each well for parametres were defined with a micro-ELISA plate reader (MR-96A, Minray, China) at a test wave-length of 450 nm. IL-2, IL-6 and IL-12 and IFN-γ concentrations in the samples were calculated by obtaining the formula from each standart regression analysis.

Microneutralization Test (mNT)

Serum virüs-neutralization test was performed according to the methods of Frey and Liess (1971). Cytopathologic effect (CPE) formation was checked every day under tissue culture microscope, and the virus titer (tissue culture infective dose 50-DKID50) at the end of the 5th day was calculated according to the Kaeber (1964) method. The presence of neutralizing antibodies against BHV-1 in blood serum samples was determined according to the mNT method reported by Frey and Liess (1971).

Statistical Analysis

Differences between groups in measured-parameters were analyzed with independent t-test, and antibody levels were analyzed with ANOVA and post-hoc Duncan tests (SPSS 10.0 for Windows/SPSS® Inc., Chicago, IL, U.S.A.). In the evaluation, $p<0.05$ was acknowledged as the significance limit.

RESULTS

Hemogram and ELISA Results

IL-2, IL-6, IL-12 and IFN-γ values were found to be significantly higher in the experimental group compared to those of the control-1 and 2 groups ($p<0.05$).

WBC values determined on day 0 were found to be high in experimental group, whereas low in control groups-1 and 2. Lymphocyte (LYM) values were detected to increased in all the animals after vaccination and iPPVO applications. IL-2, IL-6,

Table 1. Hemogram and ELISA results of blood samples taken on the 0th day (Mean ± standard deviation).

0th DAY	CONTROL GROUP 1 (n=10)	CONTROL GROUP 2 (n=10)	EXPERIMENTAL GROUP (n=20)
IL2 (ng/l)	6,1±0,64^{b}	5,5±0,38^{b}	14,8±1,38^{a}
IL6 (ng/l)	45,3±1,82^{b}	50,0±3,35^{b}	95,9±9,33^{a}
IL12 (ng/l)	2,5±0,46^{b}	1,9±0,14^{b}	3,6±0,38^{a}
IFN-γ (pg/ml)	12,0±1,09^{b}	14,0±2,61^{b}	49,5±6,42^{a}
LYM (x10^{9}/L)	5,8±0,18^{b}	4,9±0,20^{ab}	5,5±0,25^{a}
WBC (x10^{9}/L)	8,7±0,35^{b}	6,8±0,31^{a}	9,2±0,39^{a}
GRAN (x10^{9}/L)	1,6±0,17^{b}	1,0±0,97^{a}	1,4±0,10^{a}
RBC (x10^{12}/L)	5,5±0,23^{b}	5,0±0,16^{b}	5,0±0,13b
HGB (g/dl)	10,9±0,41^{b}	10,5±0,40^{b}	10,9±0,27^{b}
HTC (%)	24,8±1,21^{b}	21,8±0,81^{b}	21,9±0,47^{a}
MCHC (g/dl)	44,3±1.39^{b}	48,6±1,92^{ab}	53,5±1,42^{a}
MCH (pg)	19,8±0,35^{b}	20,9±0,59^{ab}	22,0±0,51^{a}
MCV (fl)	44,9±0,59^{b}	43,3±0,49^{a}	41,9±0,31^{c}

IL2 (Interleukin 2), IL6 (Interleukin 6), IL12 (Interleukin 12), IFN-γ (Interferon Gamma), WBC (leukocyte), LYM (lymphocyte), GRAN (granulocyte), RBC (Red Blood Cells), HGB (hemoglobin), HTC (Hematocrit). MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), MCV (Mean Corpuscular Volume). The degree of statistical significance between the groups was indicated with letters, and the presence of different letters in the same line indicates the statistical significance ($p<0.05$) between the groups.

Table 2. Hemogram and ELISA results of blood samples taken on the 4th day (Mean ± Standard deviation).

4th DAY	CONTROL GROUP 1 (n=10)	CONTROL GROUP 2 (n=10)	EXPERIMENTAL GROUP (n=20)
IL2 (ng/l)	5,9±0,73^{b}	5,4±0,78^{b}	16,9±1,13^{a}
IL6 (ng/l)	49,6±3,15^{b}	45,2±2,43^{b}	89,7±8,62^{a}
IL12 (ng/l)	1,6±0,16^{b}	1,7±0,15^{b}	2,8±0,25^{a}
IFN (pg/ml)	17,7±3,88^{b}	9,9±1,92^{b}	62,6±5,45^{a}
LYM (x10^{9}/L)	5,7±0,25^{b}	5,2±0,15^{b}	7,6±0,42^{a}
WBC (x10^{9}/L)	9,5±0,37^{b}	7,9±0,44^{b}	12,6±0,57^{a}
GRAN (x10^{9}/L)	2,4±0,34^{b}	1,5±0,28^{b}	2,1±0,24^{b}
RBC (x10^{12}/L)	5,9±0,24^{b}	4,7±0,23^{a}	5,7±0,18^{a}
HGB (g/dl)	11,8±0,37^{b}	9,9±0,33^{a}	11,6±0,26^{a}
HTC (%)	28,8±2,62^{b}	20,9±1,36^{b}	23,6±0,70^{a}
MCHC (g/dl)	44,9±2,07^{b}	48,0±1,89^{ab}	52,5±1,31^{a}
MCH (pg)	20,0±0,59^{b}	20,9±0,52^{ab}	22,4±0,45^{a}
MCV (fl)	44,8±0,73^{b}	43,7±0,66^{b}	43,5±0,46^{b}

IL2 (Interleukin 2), IL6 (Interleukin 6), IL12 (Interleukin 12), IFN-γ (Interferon Gamma), WBC (leukocyte), LYM (lymphocyte), GRAN (granulocyte), RBC (Red Blood Cells), HGB (hemoglobin), HTC (Hematocrit), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), MCV (Mean Corpuscular Volume).
The degree of statistical significance between the groups was indicated with letters, and the presence of different letters in the same line indicates the statistical significance ($p<0.05$) between the groups.

Table 3. BHV-1 Antibody Titer Values of Cattle in the Control 2 group.

BHV-1 Antibody Titer	Number of Animals (n=10)	%	Day
1/2	1	10	7th day
1/4	-	-	7th day
1/8	8	80	7th day
1/16	1	10	7th day
1/128	-	-	7th day
1/2	1	10	14th day
1/4	9	90	14th day
1/8	-	-	14th day
1/16	-	-	14th day
1/32	-	-	14th day
1/64	-	-	14th day
1/128	-	-	14th day
1/2	-	-	21st day
1/4	1	10	21st day
1/8	1	10	21st day
1/16	8	80	21st day
1/32	-	-	21st day
1/64	-	-	21st day
1/128	-	-	21st day

IL-12, IFN-γ and lymphocyte values were statistically similar in the experimental and control groups-2, while the control-1 group was different ($p<0.05$, Table 1).

When the samples taken on the 4th day in the control 1, control 2 and experimental groups were examined, it was determined that IFN-γ, IL-2, IL-6, IL-12 increased 6th hours after the 4th day of the vaccination in experimental group ($p<0.05$). WBC and lymphocyte values were similar in control 1 and 2 groups, but statistically different in the experimental group ($p<0.05$).

BHV-1 antibody titers in the blood serum of Zylexis+vaccinated animals in the experimental group; At day 7, it was defined as 2 (10%) animals at 1/2 (dilutions based on log2) and 18 (80%) animals at 1/8 titer. BHV-1 antibody titers in the blood sera of the animals in the experimental group were determined as 20 (100%) animals at 1/32 titer (log2-based dilutions) on the 14th day. BHV-1 antibody titers in the blood serum of the animals in the experimental group; On day 21, it was determined as 20 (100%) animals at a titer of 1/64 (dilutions based on log2) (Table 4).

Table 4. BHV-1 Antibody Titer Values of Cattle in the Experimental Group.

BHV-1 Antibody Titer	Number of Animals (n=20)	%	Day
1/2	2	10	7th day
1/4	-	-	7th day
1/8	18	90	7th day
1/16	-	-	7th day
1/128	-	-	7th day
1/2	-	-	14th day
1/4	-	-	14th day
1/8	-	-	14th day
1/16	-	-	14th day
1/32	20	100	14th day
1/64	-	-	14th day
1/128	-	-	14th day
1/2	-	-	14th day
1/4	-	-	21st day
1/8	-	-	21st day
1/16	-	-	21st day
1/32	-	-	21st day
1/64	20	100	21st day
1/128	-	-	21st day

BHV-1 Antibody Titer Results

The presence of BHV-1 antibodies was not detected in the blood sera of the animals in the control-1 group, which were administered only Zylexis, on the 7th, 14th and 21st days.

BHV-1 antibody titers in the blood serum of animals in control-2 group on day 7th, 1 animal (10%) at 1/2 (dilutions based on log2), 8 (80%) animals at 1/8 titer, 1/16 titer 1 (10%) animal was detected. BHV-1 antibody titers in blood serum of control group-2 animals were identified on day 14th, 1 animal (10%) at 1/2 titer (log2-based dilutions) and 9 (90%) animals at 1/8 titer. On day 21th, BHV-1 antibody titers in the blood sera of animals in the control 2 group; It was defined as 1 (10%) animal at 1/2 (dilutions based on log2) titer, 1 (10%) animal at 1/4 titer, and 8 (80%) animals at 1/16 titer (Table 3).

DISCUSSION

Infectious Bovine Rhinotracheitis (IBR) causes great economic losses. It effects nervous, respiratory and genital system in cattle, causing fertility disorders, abortions and fatal systemic diseases in newborns (Jones and Chowdhury, 2010; Raaperi et al., 2012). It has been reported that eradication and vaccination program should be initiated due to the prevalence of IBR infection in Turkiye (Alkan et al., 2018). It is known that the best way to prevent the disease is vaccination. Establishing a high antibody titer in vaccination provides long-term and safe protection. Therefore, to increase antibody titres and protectivity of vaccine, using immunomodulatory agents such as iPPVO in combination with vaccine is important. Inactive parapoxvirus ovis (iPPVO) (Zylexis®) is a nonspecific immunomodulator used to stimulate innate immunity (non-specific, innate immu-

nity) against infectious viral diseases (Schütze et al., 2009).

A study observed that in vitro phagocytic activities of peritoneal macrophages collected from mice vaccinated with iPPVO significantly increased at different time periods after vaccination. Furthermore, increased expression of IL-12 and IFN-γ, mRNA has been reported to be related to advanced in vitro and in vivo phagocytic activity of macrophages (Ons, 2014). It has been reported that intraperitoneal administration of iPPVO causes stimulation of IL 6 synthesis and fluctuations in IL-10 and IL-12 concentrations in rats (Avcı et al., 2016). In present study, increases in IFN-γ and interleukin activities were also detected.

Kyriakis et al. (1998) found that iPPVO was effective to prevent IBR infection in healthy calves kept with infected calves. Kyriakis et al. (1998) found a three-fold reduction in diarrhea incidence and a six-fold reduction in mortality between iPPVO-treated and non-iPPVO-treated piglets. The study reveals the protective feature of iPPVO against diseases. In this study, the increase in IFN-γ and interleukin activity shows that iPPVO increases protection against IBR and supports both studies.

A small pilot study in bovine herpes virus-1-infected calves showed that the use of iPPVO as an immunomodulator after the onset of an outbreak of respiratory disease can significantly help to reduce clinical manifestations in animals that may subsequently become infected (Weber, 2013). In this study, it was revealed that the use of iPPVO together with the marker IBR vaccine is beneficial for the protection of herd health.

iPPVO is also reported to induce a predominant Type 1 T helper (Th1) immune response in several species. This hypothesis is explained by the ability of iPPVO to induce immunomodulatory activity in vitro as well as in vivo (Weber et al., 2013). In this study, the increase in lymphocytes in the IBR vaccine and iPPVO groups confirms this hypothesis.

Kyriakis et al. (1998) administered iPPVO for prophylactic or metaphylactic purposes in cattle, pigs, horses, mice, dogs and cats. In the study, iPPVO proved helpful in reducing clinical findings caused by some bacterial and viral infections such as equine herpes virus, feline herpes virus, canine adenovirus type 2, Pseudomonas aeruginosa, E. coli or Pasteurella multocida, Aujeszky disease virus, IBR and vesiculitis. This effect of iPPV was claimed to be due to the stimulation of early immune mechanisms such as innate macrophage cells and increased lymphocytes induced with interferon. In our study, increased IL 2, IL 6, IL 12 and IFN-γ and lymphocyte were defined in control-1 and experimental group animals may also help to increase effectivity of IBR vaccine due to iPPVO.

Arai et al. (1990) reported that IFN-γ and IL-10 expressions were induced in animals in the experimental group after iPPVO administration in rats. In another study, IFN-γ found to increase 15-fold 6, 12 hours after iPPVO administration in rats. It was also shown that injection of iPPVO at 24th and 48th hours increased IL-12 6-fold in mice (Anziliero, 2014). In this study, IL 2, IL 6, IL 12 and IFN-γ were also found to increased in iPPVO injected groups, control-1 and experimental group. In our study, IFN gamma increased approximately 3 times in the experimental group compared to the control-1 group and approximately 6 times compared to the control-2 group at the 2nd hour. IL 12 increased 2 times. These differences between studies may be due to differences in animal species and methods used.

A study in dogs reported a marked increase in monocytes, polymorphonuclear cells and phagocytotic activity along with peripheral blood leukocytes after stimulation with iPPVO (Schütze, 2009). In this study, similar hematologic findings were found that a significant increase determined in leukocytes count in the control 1 and experimental groups, which were applied iPPVO.

In a study, it was stated that some cytokine levels increased after iPPVO application in foals and that has been suggested to be beneficial in the prevention of Rhodococcus equi infections. (Dreismann, 2010). In another study, increases in blood cytokines levels were also detected in mice and rats riceived iPPVO (Anziliero et al., 2014). It also shown that iPPVO administration increases cyrokines productions and induces an immune response in horses (Horohov, 2008). In the present study, increases in cytokine levels (IL 2, IL 6, IL 12 and IFN-γ) were defined in the control-1 and experimental group, which might be induced iPPVO.

Virus-neutralizing antibodies are virus-specific antibodies that are responsible for killing the virus. The high level of these antibodies indicates that the virus-specific humoral protection is also strong. In a study on BHV-1, ELISA and serum neutralization (SN) test were performed in healthy cattle in the Konya region, and 19% positivity in ELISA and 13% in SN test were determined, respectively (Yanbakan, 2005). Although, this study shows that there may be seropositivity in healthy animals, a similar seropositivity was not determined in the control group 1 cattle used in our study. Studies have reported that the ELISA test is more sensitive than the virus neutralization test in determining seropositivity (Duman, 2013). In the present study, virus neutralizing antibody titers were determined. It was determined only in vaccinated cattle at a titer of 1/8 in 80% of the animals on the 7th day, at a titer of 1/4 in 90% of the animals on the 14th day, and at a titer of 1/16 on the 21st day in 80% of the cattle. On the other hand, in iPPVO and vaccinated cattle, on the 7th day, 1/8 titer was in 90% of the animals, on the 14th day, 1/32 titer in all animals, and on the 21st day, the virus neutralizing antibody titer was 1/64 titer in all animals (100%) was determined. These results show that virus neutralizing antibody titers increase over time both in the vaccine group and in the vaccine and iPPVO-administered groups. However, virus neutralizing antibody titers were found to be much higher in iPPVO and vaccinated cattle, and it was observed that these titers reached up to 1/64 titers in most of the animals on the 21st day. This results indicate that administration of iPPVO and IBR vaccine simultaneously increases virus neutralizing antibody titers in cattle.

In this study, it was determined that there was an increase in cytokine concentration with the immunomodulatory effect of iPPVO with the administration of iPPVO to cattle administered IBR vaccine. It was also noted that virus neutralizing

antibody titers were significantly higher in cattle administered vaccine and iPPVO. In conclusion, iPPVO rises cytokine levels in IBR vaccinated cattle, showing the presence of immunomudulator effect of iPPVO. It was concluded that the administration of iPPVO with IBR vaccination may help to increase the effect of the vaccine. It is suggestive that administration of iPPVO and IBR vaccine simultaneously increases immun stimulation and strengthen immunoprotectivity in cattle.

CONCLUSION

In this study, it was determined that there was an increase in cytokine concentration with the immunomodulatory effect of iPPVO with the administration of iPPVO to cattle administered IBR vaccine. It was also noted that virus neutralizing antibody titers were significantly higher in cattle administered vaccine and iPPVO. In conclusion, iPPVO rises cytokine levels in IBR vaccinated cattle, showing the presence of immunomudulator effect of iPPVO. It was concluded that the administration of iPPVO with IBR vaccination may help to increase the effect of the vaccine. It is suggestive that administration of iPPVO and IBR vaccine simultaneously increases immun stimulation and strengthen immunoprotectivity in cattle.

DECLARATIONS

Ethics Approval

This study was approved by the Local Ethics Committee for Animal Care of the Burdur Mehmet Akif Ersoy University (12/12/2018- decision number: 470). Consent forms were signed by the animal owners.

Conflict of Interest

The authors declare that they have no compet of interests.

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: NM, SE

Data collection and analysis: SE, NM

Drafting of the manuscript: NM, SE

Critical review: NM, SE

Data Availability

The data used to prepare this manuscript are available from the corresponding author when requested.

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Research Article

Evaluation of cat tumors in Samsun between 2004-2022

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ABSTRACT

This study investigated the incidence of various tumors detected in 169 cat tissue and organ samples brought to the Samsun Ondokuz Mayis University, Veterinary Faculty, Department of Pathology between 2004–2022. Of the 169 specimens, five were acquired from necropsy and 164 were biopsy samples. Various tumors were evaluated retrospectively according to localization, sex, breed, and tumor origin. Tumors were common in mixed breeds (n = 96, 56.8%). Age of tumor incidence was examined; cats between 6 and 9 years of age most frequently had tumors (n = 45, 26.6%) in both sexes. In terms of tumor origin, 79 (47.0%) were mesenchymal, 83 (49.4%) were epithelial, and seven (4.2%) were of mixed components, i.e., mesenchymal and epithelial. In total, 147 (87%) tumors were classified as malignant and 22 (13%) tumors were classified as benign. Regardless of sex, the most commonly observed tumors were skin and subcutis tumors (n = 56, 32.1%). In conclusion, tumor incidence in Samsun was determined by retrospectively evaluating tumors in cats according to location, breed, sex, age range, and origin and comparing these with the literature.

INTRODUCTION

Cancer is a disease of the genome arising from DNA alterations due to mutated gene structure or function. Many agents, including viruses, chemicals, radiation damage, and altered gene expression are common features of almost all neoplasms. There is an inherent error rate in DNA replication; all multicellular organisms face the near certainty of developing a neoplasm if they survive long enough, as mutations will eventually develop (Cullen and Breen, 2017). Cats and dogs are exposed to the same environmental risk factors as humans and the complex interactions between genetic and environmental risk factors can trigger tumorigenesis. In recent years, the development of care and feeding conditions and the treatment options for various diseases has prolonged the lifespan of animals and triggered a respective increase in the frequency of neoplastic diseases in animals. In addition, the shorter lifespan of animals compared to humans causes rapid tumor progression (Cannon, 2015). Neoplastic diseases are frequently seen in domestic animals, including cats and dogs, although cats are six times less likely to develop tumors than dogs. Some dog breeds, including boxers, terriers, and bullmastiffs, are predisposed to tumor development. The predisposition of different cat breeds is controversial, but the incidence of mammary and intestinal tumors is frequently reported in Siamese cats (Egenvall et al., 2009, Egenvall et al., 2010; Rissetto et al., 2011).

Female dogs have a higher risk of developing tumors than male dogs, but this link is controversial in cats. Shida et al. (2010) reported that the frequency of tumors was substantially higher in female cats, whereas Dorn et al. (1968) observed higher frequencies in male cats. Moreover, lymphomas were more frequently seen in male cats than female cats in another study (Dorn et al., 1968) but further research found that lymphomas had no significant correlation with sex (Shida et al., 2010). In addition, one study found that the median age of tumor incidence for cats was 9.5 years old (Pérez-Enriquez et al., 2020), while another reported a median of 9.14 years old (Rafalko et al., 2022; Pinello et al., 2022b). Further research found that tumors were most often detected between 6 and 14 years of age in cats (Pamukçu, 1954; MacEwen, 1990; Gülçubuk et al., 2005; Aydın et al., 2008). Squamous cell carcinoma (SCC), mammary tumors, and fibrosarcomas are among the most frequently identified tumors in cats (Cannon, 2015).

In our country, many retrospective studies have been conducted on tumor incidence in domestic animals, including cats and dogs (Ertürk et al.,1971; Kökuslu et al., 1972; Kutsal et al., 2003; Gülçubuk et al., 2005; Atalay et al. 2007; Aydın et al.,

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2008; Kutlu et al., 2015; Kuruca et al., 2019; Aydoğan et al., 2021). This study aimed to determine the distribution of cat tumor classification according to location, tumor characteristics, sex, breed, and age of cats who attended clinics or the Samsun Ondokuz Mayis University Faculty of Veterinary Pathology Department between 2004–2022 and were examined and diagnosed by this department.

breeds, for example, orange or tabby cats (n = 96, 56.8%). In addition, six (3.6%) cats were Persian, five (3%) were Turkish Angora, and five (3%) were Turkish Van. In terms of age of tumor incidence, tumors were most common between 6 and 9 years old (n = 45 cases, 26.6%), followed by 10 to 13 years old (n = 44, 26.0%), and 1 to 5 years old (n = 34, 20.1%). There were no data available about the sex of 21 cats. The distributions of age, breed, and sex are shown in Table 1.

Table 1. Age,breed and gender incideny

Breed	Under 1 year	1-5 Age	6-9 Age	10-13 Age	14-17 Age	Unknown Age	Male	Female	Unknown Gender	General Total	Percent (%)
Mixed	-	21	25	28	8	14	29	63	4	96	56.8%
Persian	-	1	3	1	1	-	3	1	2	6	3.6%
Turkish Angora	-	1	1	2	1	1	3	3	-	5	3.0%
Siamese	-	1	2	-	-	-	2	1	-	3	1.8%
Turkish Van	-	-	2	1	2	-	3	2	-	5	3.0%
Angora-Van Mixed	-	1	1	-	-	-	1	-	1	2	1.2%
British Scottish Mixed	-	1	-	-	-	-	1	-	-	1	0.6%
British Shorthair	1	-	1	-	-	-	2	-	-	2	1.2%
Tuxedo	-	-	-	1	-	-	1	-	-	1	0.6%
Ragdoll	-	-	-	1	-	-	-	1	-	1	0.6%
Scottish	-	-	2	-	-	-	-	2	-	2	1.2%
Highlander	-	1	-	-	-	-	-	1	-	1	0.6%
Unknown	-	8	8	10	2	14	7	22	14	42	24.9%
Total (Number)	1	34	45	44	14	29	52	96	21	169	100%
Percent (%)	0.6%	20.1%	26.6%	26.0%	8.3%	17.2%	30.8%	56.8%	12.4%	100.0%	100%

MATERIALS and METHODS

The study materials originated from different ages and breeds of cats and included 164 biopsy samples and five necropsy materials. Samples were submitted to Ondokuz Mayis University Faculty of Veterinary Pathology Department from private veterinary clinics or the clinics of the Veterinary Medicine Faculty of Ondokuz Mayis University from cases recorded between January 2004 and December 2022. The samples were fixed in 10% buffered formalin solution for 48 h. Following this, the tissues were blocked in paraffin using an alcohol and xylene series, according to standard procedure for histopathological examination. Next, 5-µm thick sections were taken from the paraffin blocks, stained with Hematoxylin & Eosin, and examined under a light microscope (Nikon-Eclipse E600). Tumors were classified according to the International Histological Classification of Tumors of Domestic Animals determined by the World Health Organization (1994).

RESULTS

Tumors were most commonly seen in mixed or domestic

In female cats, 12 (12.5%) tumors were benign and 84 (87.5%) were malignant. Most benign tumors were found between 10 and 13 years old (n= 5, 15.2%), while most malignant tumors were found between 10 and 13 years old (n = 28, 84.8%). Benign and malignant tumors were mostly found between 10 and 13 years old (n = 33, 34.4%), followed by 6 to 9 years old (n = 23, 24%), then 1 and 5 years old (n = 19, 19.8%). No malignant or benign tumors were found in female cats less than 1-year-old. Most tumors were found in the mammary glands (n = 40, 41.7%), followed by the skin and subcutis (n = 22, 22.9%). No benign or malignant hemopoietic or urinary system tumors were found in female cats.

In male cats, seven (13.4%) tumors were benign and 45 (86.6%) were malignant. Benign tumors were most commonly observed in male cats between 6 and 9 years old (n = 3, 16.6%), while malignant tumors were also most often found between 6 and 9 years old (n = 15, 83.3%). Benign and malignant tumors were most commonly found between 6 and 9 years old (n = 18, 34.6%), followed by 1 to 5 years old (n = 13, 25%), and 10 to 13 years old (n = 11, 21.2%). Additionally,

most tumors were of the skin and subcutis (n = 25, 48.1%), followed by the alimentary system (n = 9, 17.3%). Mammary tumors were not found in male cats.

In terms of tumor origin, 79 (47%) were mesenchymal, 83 (49.4%) were epithelial, and seven (4.2%) were of mixed components, i.e., mesenchymal and epithelial. In total, 147 (87%) tumors were classified as malignant and 22 (13%) as benign (Figure 1). Regardless of sex, the most common tumor was of the skin and subcutis (n = 56, 33.1%), followed by mammary glands (n = 40, 23.7%), and the ears and ear canal (n = 22, 13%). The types of tumors detected in cats according to sex (male, female, and unknown), system, organ, and tissue are shown in Table 2.

Table 2. Distrubition of histopathological diagnosis for tumor according to system, organ, tissue.

System, Organ, Tissue	Histopathological Diagnose	Number of Cases By Gender			Total (Number)	Total (Percent %)
Skin and Subcutis						
		Male	**Female**	**Unknown**		
	Fibrosarcoma	12	12	3	27	16.0%
	Squamous cell carcinoma	2	7	3	12	7.1%
	Mastocytoma	3	1	-	4	2.4%
	Fibroma	2	-	-	2	1.2%
	Schwannoma	2	-	-	2	1.2%
	Lipoma	-	2	-	2	1.2%
	Liposarcoma	1	-	-	1	0.6%
	Basal cell carcinoma	-	-	1	1	0.6%
	Basosquamous cell carcinoma	-	-	1	1	0.6%
	Trichoblastoma	1	-	-	1	0.6%
	Sebaceous carcinoma	-	-	1	1	0.6%
	Feline progressive histiocytosis	1	-	-	1	0.6%
	Hemangiosarcoma	1	-	-	1	0.6%
		25	22	9	56	33.1%
Genital System						
Uterus	Leiomyosarcoma	-	1	-	1	0.6%
	Adenocarcinoma	-	2	-	2	1.2%
Ovarium	Luteoma	-	1	-	1	0.6%
	Granuloza cell tumor	-	1	-	1	0.6%
	Granuloza-Theca cell tumor	-	1	-	1	0.6%
Testis	Fibroma	1	-	-	1	0.6%
		1	6	-	7	4.1%
Mammary Gland	Cribriform carcinoma	-	15	-	15	8.9%
	Tubulopapillary adenocarcinoma	-	6	-	6	3.6%
	Tubular adenocarcinoma	-	5	-	5	3.0%

Table 2 Continue. Distrubition of histopathological diagnosis for tumor according to system, organ, tissue.

System, Organ, Tissue	Histopathological Diagnose	Number of Cases By Gender			Total (Number)	Total (Percent %)
		Male	Female	Unknown		
Mammary Gland	Fibroadenoma	-	4	-	4	2.4%
	Carcinoma and Malignant myoepithelioma	-	2	-	2	1.2%
	Ductal carcinoma	-	2	-	2	1.2%
	Adenosquamous carcinoma	-	1	-	1	0.6%
	Complex adenocarcinoma	-	1	-	1	0.6%
		-	40	-	40	23.7%
Alimentary System						
Mouth and Lips	Myxoid Liposarcoma	1	-	-	1	0.6%
	Squamous cell carcinoma	2	1	1	4	2.4%
	Fibrosarcoma	-	1	-	1	0.6%
Gingiva	Epulis	-	1	-	1	0.6%
	Fibrosarcoma	1	-	-	1	0.6%
Tongue	Squamous cell carcinoma	1	-	-	1	0.6%
Teeth	Ameloblastic fibroma	1	-	-	1	0.6%
Intestine	Leiomyosarcoma	-	1	-	1	0.6%
	B cell lymphoma	-	-	1	1	0.6%
Stomach	Gastrointestinal lymphoma	1	1	-	2	1.2%
Liver	Hepatocelluler carcinoma	-	1	-	1	0.6%
	Hepatoma	-	1	-	1	0.6%
	Mix hepatocellular and chollangiocellular carcinoma	1	-	-	1	0.6%
	Hepatic myelolipoma	1	-	-	1	0.6%
		9	7	2	18	10.7%
Respiratory System						
Nose	Poorly differentiated sarcoma	1	-	-	1	0.6%
	Nasal Adenocarcinoma	1	1	-	2	1.2%
	Fibrosarcoma	-	1	-	1	0.6%
Lungs	Papillary pulmonary carcinoma	-	1	-	1	0.6%
	Bronchoalveolar carcinoma	-	1	-	1	0.6%
	Adenosquamous carcinoma	-	1	-	1	0.6%
		2	5	-	7	4.1%

Table 2 Continue. Distrubition of histopathological diagnosis for tumor according to system, organ, tissue.

System, Organ, Tissue	Histopathological Diagnose	Number of Cases By Gender			Total (Number)	Total (Percent %)
Skeleton and Muscle System						
		Male	**Female**	**Unknown**		
Skeleton	Osteosarcoma	1	2	-	3	1.8%
	Chondrosarcoma	-	3	-	3	1.8%
		1	5	-	6	3.6%
Haematopoietic System						
Spleen	Splenic mast cell tumor	1	-	-	1	0.6%
	B cell lymphoma	1	-	-	1	0.6%
Heart	B cell lymphoma	-	-	1	1	0.6%
		2	-	1	3	1.8%
Urinary System						
Kidney	Malignant Fibrous Histiocytoma	1	-	-	1	0.6%
		1	-	-	1	0.6%
Ear and Ear Canal	Histiocytic sarcoma	1	-	-	1	0.6%
	Tubular type Adeno-carcinoma (apocrine)	1	-	-	1	0.6%
	Solar Dermal Hemangiosarcoma	1	-	-	1	0.6%
	Myofibroblastic sarcoma	-	-	1	1	0.6%
	Micropapillary type ceruminous gland carcinoma	-	1	-	1	0.6%
	Squamous cell carcinoma	3	5	5	13	7.7%
	Sebaceous epithelioma	-	-	1	1	0.6%
	Mastocytoma	-	1	-	1	0.6%
	Follicular hamartoma	1	-	-	1	0.6%
	Hemangiosarcoma	-	1	-	1	0.6%
		7	8	7	22	13.0%
Eyes and Eyelids	Rhabdomyosarcoma	1	-	-	1	0.6%
	Malign Melanoma	-	-	1	1	0.6%
	Malignant Schwannoma	-	-	1	1	0.6%
	Soft tissue sarcoma	-	1	-	1	0.6%
		1	1	2	4	2.4%

Table 2 Continue. Distrubition of histopathological diagnosis for tumor according to system, organ, tissue.

System, Organ, Tissue	Histopathological Diagnose	Number of Cases By Gender			Total (Number)	Total (Percent %)
Cardiovascular System						
		Male	**Female**	**Unknown**		
Heart and Vascular	Hemangiosarcoma	2	-	-	2	1.2%
		2	-	-	2	1.2%
Abdominal wall and abdominal cavity						
Abdomen	Malignant Fibrous Histiocytoma	1	-	-	1	0.6%
	Liposarcoma	-	1	-	1	0.6%
Omentum	Neuroendocrine carcinoma	-	1	-	1	0.6%
		1	2	-	3	1.8%
					169	100.0%

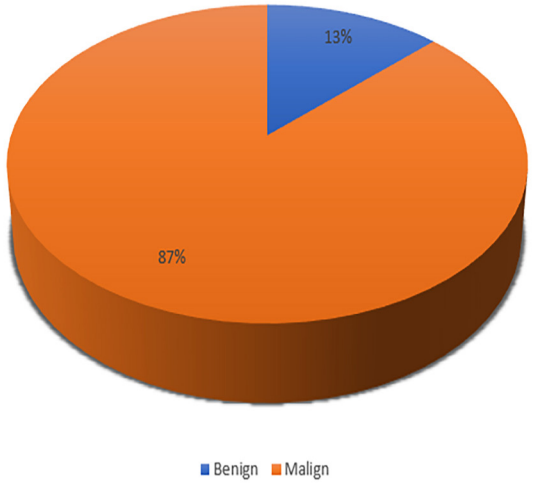

Figure 1. FeHV-1 positive cat (No. 1) with complex conjunctivitis and keratitis symptoms.

DISCUSSION

Cancer is a common cause of death for pets. However, improvements in the care and feeding of animals may lengthen their lives and increase the incidence of neoplastic diseases. Inherent DNA replication errors occur in all multicellular organisms, meaning it is almost certain that they will develop a neoplasm if they survive long enough (Cullen and Breen, 2017). Dogs develop tumors twice as frequently as humans, but cats only half as frequently. Moreover, tumor incidence depends on several variables, predominantly age, breed, sex, and geographic location (MacEwen, 1990). Various retrospective studies have been carried out on tumor incidence in domestic animals. For example, Pamukçu (1954), Ertürk et al. (1971), and Kökuslu and Akkayan (1972) identified 15 tumors over 16 years, 13 tumors from 1964 to 1970, and three tumors from 1968 to 1972, respectively. In addition, Gülçubuk et al. (2005), Vural et al. (2007), Aydın et al. (2008), and Kutlu et al. (2018) identified 132 tumors in cats between 1993 and 2004, 13 tumors from 1977 to 2005, 265 tumors from 1971 to 2005, and 394 tumors from 1985 to 2015, respectively. In our study, 169 cat tumors were found between 2004 and 2022 and were compared to other studies; the incidence of tumors in cats increased over the years. This may be due to the increased number of cats kept as pets and the increased life expectancy of such animals due to improvements in care and feeding.

The correlation between tumor incidence and cat breeds is

unclear as only Siamese cats have a reported predisposition to mammary and intestinal neoplasia (Egenvall et al., 2009; Egenvall et al., 2010). Of 132 cats with tumors examined by Gülçubuk et al. (2005), 120 (90.90%) were mixed breed and one (0.75%) was Siamese. In our study, of the 169 cats with tumors, 96 (56.8%) were mixed breed and three (1.8%) were Siamese. Of the Siamese cat tumors, one was a mammary gland tumor of the subtype tubulopapillary carcinoma, while two were skin and subcutis tumors of the subtypes schwannoma and fibrosarcoma. Although our dataset is smaller than previous studies, the most common breeds with tumors were mixed breeds in our study, which is consistent with previous findings.

The link between tumor incidence and sex in cats is controversial and has been studied several times (Kutsal et al., 2003; Gülçubuk et al., 2005; Vural et al., 2007; Aydın et al., 2008). According to Gülçubuk et al. (2005) and Aydın et al. (2008), tumors were more commonly seen in female than male cats; however Kutsal et al. (2003) and Vural et al. (2007) mostly found tumors in male rather than female cats. Our results agreed with the higher incidence of tumors in female than male cats (Gülçubuk et al., 2005; Aydın et al., 2008). Individual differences, including genetics, may trigger the formation of tumors in female cats.

Tumors are frequently observed in 6- to 14-year-old cats, with a median age of 9.1–9.5 years old (Pamukçu, 1954; MacEwen, 1990; Gülçubuk et al., 2005; Aydın et al., 2008; Rafalko et al., 2022; Pinello et al., 2022b); malignant neoplasms form a greater proportion of tumors than benign neoplasms in cats, according to Pinello et al. (2022a). Moreover, Gülçubuk et al. (2005) found malignant and benign tumors in 12 (36.36%) male cats between 6 and 10 years old and 24 (88.88%) female cats. Our data on the age range of tumor incidence in male cats is in agreement with the literature, but those on female cats are not. In this study, tumors were usually observed between 6 and 13 years of age. The mean life span of cats is between 13 and 20 years or approximately 15 years, according to several different studies (Kraft, 1998; Grimm, 2015; Cozzi et al., 2017). In addition, female cats often outlive male cats (O'Neill et al., 2015). This means that as the life span of female cats is generally longer than male cats, tumors may be observed in female cats at a higher ratio.

Various studies on tumor frequency in cats have been published (Gülçubuk et al., 2005; Shida et al., 2010; Manuali et al., 2020). Shida et al. (2010) observed that the skin and subcutis were the most common sites for tumors (n = 318, 29.7%), followed by the mammary glands (n = 182, 17.0%), and the lymphoid/hematopoietic system (n = 176, 16.5%). Manuali et al. (2020) found that skin and soft tissue tumors constituted 55.9% of all tumors, followed by mammary gland (11%) and alimentary tract (7.9%) tumors. In contrast, Gülçubuk et al. (2005) reported that mammary tumors were found in 46 cats (34.84%) regardless of sex, followed by 22 (16.16%) skeletal and muscle system tumors, and 20 (15.90%) skin and subcutis tumors. Our results were similar to Manuali et al. (2020).

Skin neoplasms generally comprise fibrosarcomas, SCCs, and basal cell tumors (Ho et al., 2018; Manuali et al., 2020). In our study, regardless of sex, the most common tumor was fibrosarcoma (n = 27, 16%), followed by SCC (n = 12, 7%), and mastocytoma (n = 4, 2.4%). However, injection, vaccination, and microchip implantation frequently cause injection site sarcomas in cats (Carminato et al., 2011; Martano et al., 2011; Martano et al., 2012; Dean et al., 2013). In our study, fibrosarcomas related to injection or vaccination were commonly observed at the injection site. In recent years, vaccinations, microchip implantation, and injections have increased because the number of cats being adopted has increased; this may have caused an increase in the incidence of fibrosarcoma in cats.

Although mammary tumors are among the most common tumors in female cats, they have also been reported in male cats (Skorupski et al., 2005). In one study, the highest tumor rates were observed in cats aged 10–11 years old (Dorn et al., 1968), whereas Gülçubuk et al. (2005) detected the highest rate of mammary tumors between 8 and 12 years old. Malignant tumors were found more frequently than benign tumors and consisted of different adenocarcinoma subtypes (Pamukçu, 1954; Kökuslu and Akkayan, 1972; Gülçubuk et al., 2005; Aydın et al., 2008; Mills et al., 2015). Therefore, our results were in agreement with the literature (Pamukçu, 1954; Kökuslu and Akkayan, 1972; Gülçubuk et al., 2005; Aydın et al., 2008; Mills et al., 2015). Hormonal changes during the estrous cycle or pregnancy could potentially trigger tumor development in mammary glands.

In the literature, the incidence of epithelial tumors is higher than that of mesenchymal tumors (Goldschmidt and Goldschmidt, 2016). Pamukçu (1954) analyzed 15 cat tumors, of which eight (53.33%) were of epithelial and seven (46.66%) were of mesenchymal origin. Similarly, Ertürk et al. (1971) reported 13 cat tumors, of which six (46.15%) were epithelial and five (38.4%) were mesenchymal in origin, as well as two (15.38%) teratomas. Kökuslu and Akkayan (1972) detected three (100%) tumors in cats of epithelial origin. Moreover, Aydın et al. (2008) observed 265 tumors; 134 (50.57%) were epithelial and 131 (49.43%) were mesenchymal in origin. Kutlu et al. (2015) detected 14 tumors in cats; nine were epithelial and five were of undefined origin. Gülçubuk et al. (2005) examined 67 mesenchymal (50.75%), 60 (45.45%) epithelial, and five (3.78%) mixed tumors. Our data are similar to the literature, except for Gülçubuk et al. (2005). However, many factors, including genetics, individual differences, and aging, may stimulate epithelial-mesenchymal or mesenchymal-epithelial transition in addition to causing tumors of different origins.

MacVean et al. (1978) reported that the incidence of feline lymphoma was 168 per 100,000 cats, which represented 41% of feline neoplasms; the sex-specific rate was higher for males than for females. Moreover, the risk of developing lymphoma in male cats was two to three times that of female cats (Dorn et al., 1968). Mooney et al. (1989) examined 103 lymphoma cases over the course of four years, while Shida et al. (2010) found 132 lymphoma cases over 23 years. Kutlu et al. (2018) recorded lymphoma in 10 cats over 31 years, of which five were male and four were female, and determined that three lymphomas were located in the intestine, two in the skin, and five in other organs and tissues. Gülçubuk et al. (2005) detected a total of three lymphomas over 11 years, two of which

were in the small intestine, that is, alimentary lymphoma, and one was located in the lymph node; two cats were of unknown sex and one was female. In the current study, five lymphoma cases were found over 18 years; two (1.2%) tumors were in the intestine, one (0.6%) in the stomach, one (0.6%) in the spleen, and one (0.6%) in the heart. Lymphomas were less frequently reported in our country than in other countries (Mooney et al., 1989; Shida et al., 2010; Kutlu et al., 2018). This may be because fewer cats are kept as pets in our country than in other countries. Alternatively, cats may not receive the same level of veterinary care and therefore samples may not be sent to pathology laboratories, such as biopsy, necropsy, or cytology samples.

CONCLUSION

In this study, a significant increase in several tumor types was observed, including skin and mammary tumors, in cats from 2004 to 2022. We hypothesize that this was due to improvements in the care and feeding of the animals, the prolongation of their life span, the increase in the number of patients brought to the clinic, and the increased number of animal owners.

DECLARATIONS

Ethics Approval

Not applicable.

Conflict of Interest

The authors declared that there is no conflict of interest.

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: MTK, TG

Data collection and analysis: MYG,TG, MY, MS, YBK, EK,Sİ, NK, MTK, FBA

Drafting of the manuscript: MTK,TG

Critical review: TG

Data Availability

The author has provided the required data availability statement.

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Research Article

Feline herpes virus-1 (FeHV-1) in cats with ophthalmic problems: attempted propagation in CRFK cell lines

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ABSTRACT

Feline herpesvirus-1 (FeHV-1) is classified within the Varicellovirus genus and is frequently seen in cats. Ocular complications, such as conjunctivitis, keratitis, and corneal ulcers, are common and have the potential to result in latency and permanent visual loss if not appropriately diagnosed and monitored. This study aimed to isolate FeHV-1 from cats with ocular lesions using the CRFK cell line. This study included a total of ten cats that tested positive for FeHV-1 using PCR and showed symptoms, including ocular and nasal discharge (8/10), conjunctivitis (6/10), and keratitis (5/10). Conjunctival samples were collected and processed for nucleic acid extraction. The CRFK cell line was propagated, and all positive samples were inoculated in 6-well plates. Interestingly, no CPE was observed in the CRFK cell cultures during the observation period. Following post-inoculation in cell culture, the PCR analysis conducted on the supernatants obtained from the cultures found negative for FeHV-1. This study points out the challenges faced in isolating FeHV-1 in the CRFK from ocular samples of naturally infected cats. This highlights the requirement for future comprehensive in vitro studies to enhance the efficacy of FeHV-1 isolation techniques and explore potential approaches for FeHV-1 diagnosis.

INTRODUCTION

Feline viral rhinotracheitis (FVR) can manifest itself clinically in a variety of ways, including sneeze, nasal discharge, ocular discharge, conjunctivitis, coughing, oral ulcers, anorexia, fever, and lethargy (Gaskell et al. 2007; Magouz et al., 2022; Stiles 2014). *Feline herpesvirus type 1* (FeHV-1), *feline calicivirus* (FCV), *Chlamydia felis*, and more recently, *Bordetella bronchiseptica* (*B. bronchiseptica*) and *Mycoplasma felis* (*M. felis*) are the most common pathogens related with FVR (Lister et al., 2015; Sykes and Shelley, 2013; Walter et al., 2020).

FeHV-1 is a significant viral pathogen that affects domestic cats worldwide. FeHV-1 is a DNA virus, belongs to the genus *Varicellovirus*, subfamily *Alphaherpesvirinae*, and family *Herpesviridae* and is the primary causative agent of FVR, a respiratory disease characterized by sneezing, nasal discharge, and ocular manifestations (Gaskell et al., 2007). It has also been reported that clinical symptoms such as fever, oculo-nasal discharge, conjunctivitis, keratitis, pneumonia, abortion and fetal death can be seen in cats in the infection caused by FeHV-1 (Lee et al., 2019). Ocular complications associated with FeHV-1 infections, such as conjunctivitis, keratitis, and corneal ulcers, are common and can lead to severe visual impairment if not appropriately managed (Stiles, 2014).

Cats with acute FVR may shed the agent from and any age, vaccinated or unvaccinated, can be occasionally affected by this infection (Povey, 1979; Sykes et al., 1997). Most infected cats will later go on to become carriers because of the infection's tendency to remain latent in the neural ganglia (Haid et al., 2007). FeHV-1 can remain latent especially in the tissues of the head (trigeminal ganglia, optic nerves, olfactory bulbs, cornea, throat, salivary gland, lacrimal gland, cerebellum and conjunctiva) (Burgesser et al., 1999).

Virus isolation in cell culture, pathological findings, direct immunofluorescence test, PCR and serological methods are used in the diagnosis of FeHV-1 (Lister et al., 2015; Sykes and Shelley, 2013; Tan et al., 2020). One of the most effective ways among these methods is the isolation of the agent in a susceptible cell culture. The CRFK (Crandell-Rees feline kidney) cell line is widely used for propagating various feline viruses, including FeHV-1, due to its susceptibility to infection and ability to support viral replication (Haid et al., 2007; Henzel et al., 2011). CRFK cells are derived from feline kidney tissue and therefore provide an environment that is more physiologically relevant to FeHV-1 compared to non-feline cell lines. This natural host compatibility can enhance viral replication and allow for the study of specific virus-host interactions. The cells maintain their susceptibility to FeHV-1 over extended

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periods, allowing for long-term studies and the establishment of persistent infections. CRFK cells provide an excellent platform for studying FeHV-1-induced cytopathic effects (CPEs) and viral morphogenesis (Gaskell et al., 2007). The cells readily support viral replication, leading to characteristic changes in cell morphology that can be visualized and analyzed using various microscopy techniques. As a consequence of long-term cultivation in vitro, CRFK cells may undergo genetic changes and acquire phenotypic alterations. These changes can influence the susceptibility to FeHV-1 infection, alter the expression of host factors, and potentially impact the interpretation of experimental results (Maes, 2012).

In this regard, ocular samples taken from cats with ophthalmic lesions were inoculated into CRFK. It was aimed to produce FeHV-1 by inoculating the inoculum prepared from the samples taken for our research into sensitive cell culture. For effective treatment methods and preventative measures against ocular disorders in cats, it is crucial to understand the mechanism of action and replication of FeHV-1 in CRFK.

MATERIALS and METHODS

Animals

Ten FeHV-1-positive cats of varying ages, breeds, and genders with ocular lesions were used in this study between 2021 and 2022. They were brought to the Virology Laboratory of Veterinary Medicine, the Animal Hospital of Burdur Mehmet Akif Ersoy University, and private veterinary clinics. Veterinarians of the patients provided information on the cats to which each sample belonged. Animals had a variety of severe symptoms, including oculo-nasal discharge, conjunctivitis, and keratitis. The same veterinarian conducted an ophthalmological examination on each animal and recorded its symptoms.

Samples

The samples were collected with sterilized commercial swab sticks dipped in antibiotic PBS. All of the liquid in the swab sticks was transferred to sterile 2 ml microtubes after entirely mixing with a vortex. It was centrifuged for 20 minutes at +4 °C at 3000 rpm. After centrifugation, 500 µl of the supernatant was taken and kept at -80 °C until tests.

Nucleic acid extraction and PCR test

The resulting supernatants were extracted with a commercial viral nucleic acid isolation kit (Roch, Germany). Post-extraction PCR test was performed in accordance with the method of Henzel et al., (2012). Samples found to be positive were aliquoted and stored at -80 °C until tests. An extract from a commercial vaccine that contains FeHV-1 was used as the positive control in the PCR test. As negative control ultrapure water was used.

Cell culture

In this study, a CRFK cell line susceptible to feline viral infections was used. As a cell culture maintenance medium, sterile commercial Dulbecco's Modified Eagle Medium (DMEM) with high glucose (4.5 g/l) was used. Prophylaxis included antibiotics (10000 units/ml penicillin, 10.00 mg/ml streptomycin, 9.00 mg/ml sodium chloride) and antimycotics (250 g/ml amphotericin B, 205 g/ml sodium deoxycholate). Sterile fetal calf serum (FDS) was used at a rate of 10% for cell culture research. CRFK cell cultures were propagated in 25 cm^2 (50 ml) and 75 cm2 (250 ml) flasks (Corning, USA). Cell growth media was sterile commercial DMEM High Glucose (4.5 g/l) with L-Glutamine containing 10% FDS, 10% antibiotic, and 7% antimycotic. The cells cultures were incubated at 37°C in a humidified 5% CO^2 atmosphere for 3-4 days before being used for experiments. The incubated cells were inspected daily using an inverted tissue culture microscope (Olympus, Japan).

Inoculation of positive samples in CRFK

Cells that had been propagated were transferred into 6-well plates. Pre-conserved samples were thawed immediately in a 37 °C water bath. On two plates, ten samples were inoculated. After passing through 0.22 m sterile filters, all samples were inoculated into cell culture. Each plate had a cell control (HK) well. The same volume of PBS as the inoculum was given to the cell control wells. Every well was tested daily for any signs of cytopathologic effects (CPE) for 5 days after inoculation. All plates were freeze-thawed at -80 and 37 °C at the end of the fifth day. All well fluids were transported to 15 ml centrifuge tubes. All tubes were centrifuged for 20 minutes at +4 °C at 3000 rpm. 2 ml of supernatant was taken from each tube for PCR testing. All samples were blind passaged twice in CRFK.

RESULTS

Symptoms

Ten FeHV-1-positive cats showed signs of oculo-nasal discharge (8/10), conjunctivitis (6/10), and keratitis (5/10), either individually or in multiple combinations (Table 1) (Fig 1).

Cell culture

Table 1. Clinical signs of cats with FeHV-1 positive.

Symptoms	Numbers	Percentage	Total
Oculo-nasal discharge	8	%80	10
Conjunctivitis	6	%60	10
Keratitis	5	%50	10

Frozen samples were allowed to thaw in a 37 °C water bath for maximum progress. Both 6-well plates were seeded with the CRFK cell line. A single well on each plate was assigned as a cell control. However, there were no cases of CPE detected within the observed time period, although all samples were blind-passed twice in CRFK (Fig 2).

Post-inoculation (PI) PCR test

A total of ten positive swab samples were seeded onto a six-well plate that had been coated with CRFK. After five days PI, the supernatants from those cultures were collected and used for DNA extraction. We used the same PCR method described in the materials and methods section to check these samples for FeHV-1 nucleic acid. There were no positive results seen in any of the samples. (Fig 3).

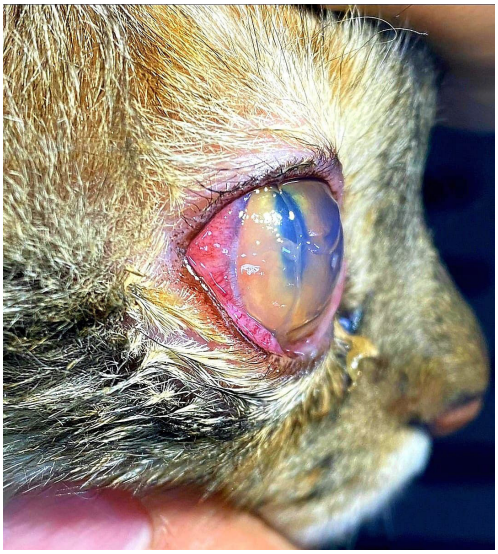

Figure 1. FeHV-1 positive cat (No. 2) with complex conjunctivitis and keratitis symptoms.

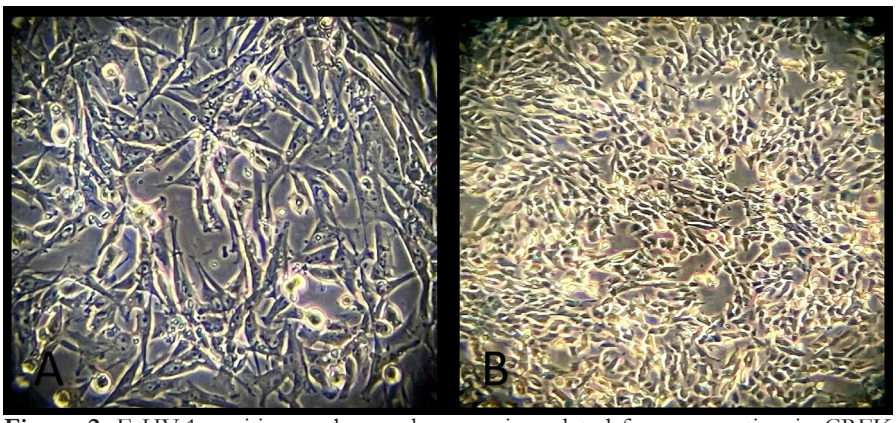

Figure 2. FeHV-1 positive swab samples were inoculated for propagation in CRFK cell lines. No CPE were detected. (A) Control, uninfected CRFK cells at 24 h. 10x magnification (B) CRFK cells inoculated with sample no: x at 120 h post-inoculation. 4x magnification. Olympus CKX41 inverted microscope (Olympus Corporation, Tokyo, Japan).

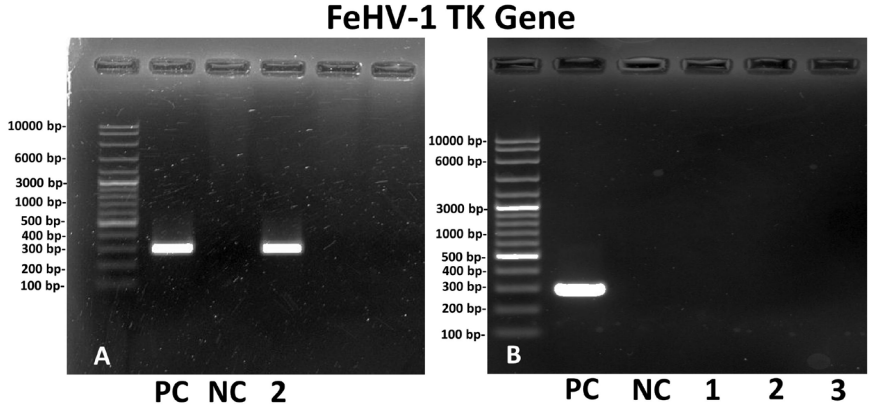

Figure 3. A conducted PCR test result before and after being inoculated, shown in the gel electrophoresis image. The swab sample collected from cat No. 2, which exhibited clinical symptoms including noticeable discharge from the eyes and nose as well as conjunctivitis, yielded a positive result prior to inoculation (A. before to inoculation; B. after inoculation). PC: FeHV-1 contained vaccine extract. NC: Ultrapure water.

DISCUSSION

FeHV-1 infects cats leading to in a wide range of clinical signs, including but not limited to sneeze, nasal discharge, ocular discharge, conjunctivitis, coughing, oral ulcers, anorexia, fever, and lethargy (Stiles, 2014). PCR detection of FeHV-1 can be routinely performed in oculo-nasal secretions from conjunctival or nasopharyngeal swab, and in uncoagulated blood (Walter et al., 2020). In most cases, these samples should be tested at the same time since their integrated results can be useful in the diagnosis of FeHV-1 infection (Sykes and Shelley, 2013). For pharynx, tongue, nose and conjunctiva samples, a recent study has shown that FeHV-1 tended to be most frequently detected in the nose, but this difference was not significant compared with other locations (Schulz et al., 2015). FeHV-1 infection causes acute upper respiratory and ocular disease, which is particularly severe in kittens. The tonsils, conjunctiva, and nasal mucosa are the locations where FeHV-1 predominantly infects muco-epithelial cells (Gould, 2011). So, oronasal and conjunctival swab extracts are often used as samples for virus isolation or PCR to diagnose acute FeHV-1 in the lab. It is thought that corneal dendritic ulcers are the pathognomonic sign of FeHV ocular infection (Maes, 2012). Almost all cats experience a latent infection following the acute phase of the disease, which can occasionally reactivate and result in recurring clinical symptoms, mostly ocular infections like keratitis and conjunctivitis. Hence, the use of conjunctival swabs has significant value in the detection and isolation of FeHV-1 (Henzel et al., 2011; Litster et al., 2015). This study included cats with a high prevalence of oculo-nasal discharge (80%), however because of their anatomy, we were only able to collect the conjunctival swab samples. This has also been highlighted as a concern by previous research (Veir et al., 2008).

Traditional methods involve the inoculation of clinical samples onto susceptible cell cultures, followed by observation for CPE. Low viral loads can present challenges for virus isolation as they may not contain sufficient infectious viral particles to establish productive infection in cell cultures. Despite the detection of viral nucleic acids through PCR-based assays, the presence of low viral load can lead to the failure of virus isolation. Studies have shown that samples with low viral loads often exhibit weak or no CPE in cell cultures, limiting the ability to isolate infectious virus (Leland and Ginocchio, 2007). Accurate quantification of viral load in clinical samples could help determine the likelihood of successful virus isolation. Cats with eye problems were included in our study, however it is possible that the swab samples we collected did not contain enough infectious viral particles to be propagated in CRFK cell culture. The likelihood of successfully isolating the virus might be improved by using a procedure involving two or more blind passages (Haid et al., 2007). There are a few reasons why PCR is a more sensitive method for identifying FeHV-1 in oculo-nasal secretions than traditional tissue culture isolation methods (Burgesser et al., 1999; Sandmeyer et al., 2010). This could also be an explanation of why we were unable to grow virus in CRFK in our study, although all samples detected positive by PCR. Transport, freezing, thawing, and the enzymes in saliva and tears might have all been involved in the decomposition of the herpesvirus envelope. It's possible that the antibodies complexed with the virus in the tears and saliva, reducing the virus's infectivity. It's also probable that many of the infectious virions released in the saliva and tears are defective in maturation (Reubel et al 1993).

The cell culture method used is crucial for effective virus isolation, for FeHV-1 isolation depends on the specific strain or isolate being studied. Different cell lines have been used for successful FeHV-1 isolation, and their suitability may vary (Leeming et al., 2006; Sun et al., 2014; Yang et al., 2020). CRFK cells, derived from feline kidney tissue, are one of the most widely used cell lines for FeHV isolation (Walter et al., 2020). In our study, the failure to isolate FeHV-1 was unexpected; there is a well-described carrier state in FeHV-1 infections, and they appear to be widespread. Permanent cell lines used for viral propagation may not always support the replication of a given virus strain. Some strains have strict tropism and may require specific cell types expressing appropriate receptors for productive infection (Yang et al., 2020). If the selected cell line lacks the necessary receptors or host factors, the virus may fail to establish infection, despite the detection of viral nucleic acids in the clinical sample. FeHV-1 exhibits a natural tropism for epithelial cells, particularly those of the respiratory tract (Leeming et al., 2006). However, in some cases, FeHV-1 may establish a non-productive or latent infection in cell culture systems, leading to the absence of observable CPE (Cannon et al., 2010). This non-productive infection can hinder successful virus isolation, as it becomes difficult to distinguish between true virus isolation failure and latent infection. On the other hand, while other pathogens such as *B. bronchiseptica* have the potential to be a primary agent, they are more likely to contribute to FVR as a secondary or opportunistic infection in the clinical setting (Sykes and Shelley, 2013; Walter et al., 2020). We had to abandon comprehensive pathogen identification for this study due to a lack of resources. The results could indicate an uncommon FeHV-1 infection in cats, but further work is necessary to confirm these preliminary findings.

Cellular factors present in the clinical sample may inhibit viral replication, thereby preventing the successful isolation of infectious virus. Various components such as antiviral proteins, low pH, or lack of some essential amino acids can negatively impact FeHV-1 replication in CRFK (Maggs et al., 2000; Storey et al., 2002). These factors may not be adequately accounted for during PCR-based detection, leading to the false-positive result without successful virus isolation. In our research, we used commercial DMEM supplemented with arginine and lysine at 84 and 146 mg/L, respectively. By optimizing these amino acid concentrations in this medium, positive results may be obtained for isolating FeHV-1 in CRFK (Bol and Bunnik, 2015).

Virus isolation is a crucial step in understanding the pathogenesis, epidemiology, and potential therapeutic interventions for viral infections. Feline viruses have long been studied and isolated using cell culture techniques (Sykes and Shelley, 2013). Although cell culture techniques are widely used, viral adaptation needs virus isolation from persistent cell cultures, which has shown to be challenging or unsuccessful (de Parseval et al., 2004). Virus culture, isolation, and identification of FeHV-

1 from pharynx, tongue, nose and conjunctiva or buffy coat samples is strongly supportive of a diagnosis of FVR in cats with compatible clinical signs. The duration for virus culture and identification in a laboratory can be as minimal as 4-5 days when the sample has an adequate viral load. However, due to the lengthy procedure of tests completion, these tests are of limited practical use in rapid diagnosis of disease.

CONCLUSION

The objective of the study was to successfully and rapidly isolate FeHV-1 from cats that were naturally infected and showed eye problems. In light of unexpected results, our investigation focused at exploring the factors associated with struggling to isolate FeHV-1 in the CRFK cell line. Finally, we tried to clarify the potential underlying causes behind the limitations. In conclusion, further studies are necessary in in vitro systems to effectively isolate FeHV-1 and exploring potential control and treatment strategies.

DECLARATIONS

Ethics Approval

All procedures were approved by the Animal Ethics Committee (AEC) Burdur Mehmet Akif University, Türkiye (No:102/914).

Conflict of Interest

Authors do not have any conflict of interests

Consent for Publication

Consent on publication was comfirmed with approval from the Republic of Türkiye Ministry of Agriculture and Forestry, Directorate of Burdur Provincial (No: E-69877819-325.04.02-6073614).

Competing Interest

The authors declare that they have no competing interests

Author contribution

Idea, concept and design: HSS, YF

Data collection and analysis: HSS, YF

Drafting of the manuscript: HSS

Critical review: HSS

Data Availability

Not applicable.

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Research Article

The effect of oral administration of robenacoxib on hematological and biochemical parameters in different goat breeds

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ABSTRACT

Robenacoxib is a coxib-class, highly selective cyclooxygenase-2 inhibitor that is used to control pain and inflammation. This study aimed to determine the effect on hematological and biochemical parameters 24 hours after oral administration of robenacoxib to healthy Alpine and Saanen goats. The study was conducted on healthy females, 1-2 years old Alpine (n=5) and Saanen (n=5) goats. A single dose of robenacoxib was administered orally to goats at a dose of 4 mg/kg body weight. Blood samples were taken before (0 hour) and 24 hours after the administration of robenacoxib to evaluate of hematological and biochemical changes. The results of this study showed that statistical differences in hematological and biochemical parameters were within the normal limits in Alpine and Saanen goats except lactate dehydrogenase in Saanen goats. Furthermore, no differences were observed in hematological parameters between goat breeds; it can be claimed that the Alpine breed is more susceptible to negative pharmacological side effects than the Saanen breed according to changes in biochemical parameters. As a result, it was concluded that robenacoxib did not have a negative effect on kidney and liver functions and blood components in Alpine and Saanen goats in the administered dose and treatment period in this study. Further studies need to investigate the effects of robenacoxib in high doses, long-term use, and in disease conditions, especially on the kidney.

INTRODUCTION

Changes in moral and ethical considerations have led to global demands for better welfare conditions for food animals and the need for better agricultural practices. From this perspective, controlling pain sensation is one of the critical parameters necessary for improving livestock welfare. In addition to improving animal welfare standards, analgesia is also necessary both to facilitate procedures and to improve animal and personnel safety (Smith et al., 2021).

Goats are very pain-sensitive animals and cannot tolerate pain-inducing procedures. In the postoperative period, sudden deaths have been reported in goats, which have been attributed to catecholamine-induced ventricular fibrillation resulting from inadequate analgesia (Gray and McDonell, 1986). Chronic pain without relief produces significant stress and changes in behavior, as well as cardiopulmonary, neuroendocrine, metabolic, immunological, and thermoregulatory disorders (Anderson and Muir, 2005).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used in veterinary and human medicine due to their anti-inflammatory, analgesic, and antipyretic effects. NSAIDs act by inhibiting various cyclooxygenase (COX) enzyme isoforms that play a role in the synthesis of prostaglandins, which have important efficacy in inflammatory and physiological events (Gunaydin and Sirri Bilge, 2018). Non-inducible COX-1, which is expressed by many body tissues, stimulates prostaglandin production for beneficial effects in the gastrointestinal tract, kidneys, nervous, reproductive, and cardiovascular systems (Clark, 2006). On the other hand, COX-2 is induced locally and for a limited time and is mainly responsible for inflammation and pain (Morita et al., 1995). Therefore, NSAIDs with a broad therapeutic index (selective COX-2 inhibitors, COXIBs) have been developed (Flower, 2003). Robenacoxib, one of the COXIBs that selectively inhibit COX-2, has been marketed as injectable and tablet formulations for cats and dogs (EMA, 2008). Animal species other than cats and dogs could potentially benefit from this drug.

There are no NSAIDs approved for use in the treatment of pain in small ruminants in Türkiye, USA, and Europe (Smith et al., 2021; Traş et al., 2021). As a result, these drugs are used off-label. Depending on the use of off-label drugs, treatment failure, drug-related adverse effects, a decrease in the production of healthy and quality animal product and economic losses may occur. Changes in hematological and biochemical parameters can be observed as a result of drug-related adverse effects (Amin et al., 2017; Owumi and Dim, 2019).

Hematological and biochemical parameters are considered indicators of structural toxic effects (Thrall, 2004). The effect of robenacoxib on hematological and biochemical parameters in goats has not been reported so far. In addition, studies have shown that there are differences among goat breeds concerning their hematological and biochemical profiles (Azab and Abde-Maksoud, 1999; Tibbo et al., 2004). Alpine and Saanen breeds generally have high adaptability and strong constitutions. One of the breeds' most important characteristiscs is their quick adaptation to different climatic conditions. Their

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feed utilization ability is high (Gall 1996). Because of these features, Alpine and Saanen goats were chosen in this study, which are raised almost all over the world. The purpose of this study is to investigate whether robenacoxib affects hematological and biochemical markers in different goat breeds (Alpine and Saanen) at 24 hours (24-h) after an oral single dose administration.

MATERIALS and METHODS

Animals

The study was carried out on a total of 10 goats of Alpine (n=5) and Saanen (n=5) breeds, 1-2 years old, 25-35 kg in weight, determined to be healthy by general clinical examination. The animals did not receive any other medications for at least 2 months prior to the beginning of this study. Goats according to breed, were kept in separate compartments from the other animals on the farm during the study. The goats were fed drug-free commercial feed twice a day and had free access to dried alfalfa grass and water. The experimental procedures in this study were approved by the Ethics Committee of the Cukurova University, Health Sciences Experimental Application and Research Center. This study was carried out at the Dairy Goat Research Farm, Faculty of Agriculture, Cukurova University, Adana, Türkiye.

Experimental Design

A single dose of robenacoxib (40 mg/tablet, Onsior®, Elanco) was administered orally at a dose of 4 mg/kg (Fadel et al., 2023) to goats. During the study, the animals were clinically observed. Before (0 hour, control) and at the 24-h after the administration, 3 mL of blood samples were taken with a vacutainer into tubes with K_3EDTA for hematological analyses and without anti-coagulant for biochemical analyses. Hemotological measurements were performed immediately after blood collection. The blood samples taken for biochemical measurement were centrifuged for 10 minutes (2500 ×*g*), and the serum samples obtained were stored at -20 °C until the analysis and analyzed within a week.

Analysis of Hematological and Biochemical Parameters

Measurement of hemotological parameters from blood samples taken into EDTA tubes (white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), neutrophils (Neu), lymphocytes (Lym), monocytes (Mon), eosinophils (Eos), basophils (Bas), neutrophils% (Neu%), lymphocytes% (Lym%), monocytes% (Mon%), eosinophils% (Eos%), and basophils% (Bas%)) were measured in a blood counter (Mindray BC-5000 Auto Hematology Analyzer, Mindray Bio-Medical Electronics, Shenzhen, China). Measurement of biochemical parameters (blood urea nitrogen (BUN), creatinine (CRE), total protein (TP), albumin (Alb), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), lactate dehydrogenase (LDH), and creatine kinase (CK)) from serum samples was performed in an autoanalyzer device (Beckman Coulter AU 5800, Indianapolis, United States).

Statistical Analysis

Hematological and biochemical parameters were presented as mean ± SD. The SPSS program (23.0 software; IBM) was used for statistical analysis. The data obtained in the study were evaluated with the paired-t test. A value of $p < 0.05$ was accepted as the limit of statistical significance.

RESULTS

The current study investigated the effects of a single oral dose of robenacoxib on heamotological and biochemical parameters in Alpine and Saanen goat breeds. After oral administration of robenacoxib at a single dose of 4 mg/kg, no difference was detected in the feed and water consumption, rumination, defecation, urination, and movements of the goats during clinical observations. The findings indicated that a single oral dose of robenacoxib after 24-h treatment significantly altered some hematological and biochemical parameters within the reference range in both goat breeds (Table 1, Table 2, $p < 0.05$).

Alterations in Hematological Parameters

The effects of robenacoxib on hematological parameters in Alpine and Saanen goats were presented in Table 1. Robenacoxib showed no significant effect on WBC, HGB, MCV, Lym, Eos, Bas, Eos%, and Bas% after the 24-h treatment period when compared with the 0-h in the Alpine goat breed ($p < 0.05$; Table 1). While robenacoxib treatment significantly decreased RBC, HCT, Neu, and Neu%, MCH, Mon, Lym%, and Mon% were significantly increased after the 24-h treatment period when compared with the 0-h in Alpine goats ($p < 0.05$; Table 1). The obtained data revealed non-significant alteration in the WBC, HGB, Neu, Lym, Bas, Neu%, Eos%, and Bas% after the 24-h treatment period when compared with the 0-h in Saanen goats. However, robenacoxib had a significant effect on RBC, HCT, MCV, MCH, Mon, Eos, Lym%, and Mon% after the 24-h treatment period in this goat breed ($p < 0.05$; Table 1). The results indicated that there are significant decreases in RBC, HCT, MCV, and Lym% at 24-h treatment period in the Saanen goats ($p < 0.05$; Table 1). Additionally, MCH, Mon, Eos, and Mon% were significantly increased in the robenacoxib treated group at the 24-h treatment period in the Saanen goats when compared with the 0-h treatment period ($p < 0.05$; Table 1).

Alterations in Serum Biochemical Parameters

Robenacoxib effects on biochemical parameters in Alpine and Saanen goats were presented in Table 2. Robenacoxib had no significant effect on CRE, TP, Alb, and CK after the 24-h treatment period when compared with the 0-h in Alpine goats. The biochemical profiles, such as serum BUN levels and GGT enzyme activity were significantly increased, whereas serum AST, ALT, and LDH enzyme activities were significantly decreased at 24-h treatment period in Alpine goats ($p < 0.05$; Table 2). Robenacoxib significantly affected serum GGT and LDH activities after the 24-h treatment period when compared with the 0-h in the Saanen goats ($p < 0.05$; Table 2). Serum GGT and LDH activities were increased in robenacoxib treated Saanen goats at 24-h ($p < 0.05$; Table 2).

Table 1. Effects on hematological parameters (mean ± SD) after oral administration of a single dose of robenacoxib (4 mg/kg) to Alpine and Saanen goats (n=5)

Hematological Parameters	Alpine		Saanen		Reference Range
	0-h	24-h	0-h	24-h	
WBC (×10^9/L)	14.53±2.82	13.56±2.04	14.20±3.34	15.37±4.00	5.80-25.00
RBC (×10^{12}/L)	18.37±2.71	14.34±2.22*	15.53±0.72	14.46±0.81**	10.00-21.00
HGB (g/dL)	9.78±0.90	8.46±0.84	8.68±0.80	8.70±0.85	6.2-13.5
HCT (%)	24.66±1.64	19.22±1.46*	21.90±1.58	20.04±1.53**	19.00-36.00
MCV (fL)	13.58±1.40	13.54±1.34	14.08±0.62	13.84±0.53**	13.00-23.00
MCH (pg)	5.38±0.35	5.94±0.38*	5.58±0.33	6.02±0.36**	4.2-7.8
Neu (×10^9/L)	7.23±1.89	5.78±1.39*	6.41±2.51	6.95±2.20	2.12-10.10
Lym (×10^9/L)	6.86±1.18	7.05±1.36	7.37±1.32	7.59±2.32	3.12-22.10
Mon (×10^9/L)	0.08±0.03	0.32±0.14*	0.13±0.05	0.39±0.11**	0.00-1.42
Eos (×10^9/L)	0.31±0.27	0.36±0.12	0.21±0.09	0.34±0.08**	0.00-1.32
Bas (×10^9/L)	0.06±0.02	0.06±0.02	0.08±0.03	0.10±0.03	0.00-0.35
Neu %	49.18±7.21	42.4±8.23*	44.36±6.97	44.96±6.30	13.0-58.0
Lym %	47.92±7.52	52.22±8.78*	52.70±7.17	49.52±6.75**	35.0-83.0
Mon %	0.50±0.12	2.30±0.93*	0.86±0.11	2.58±0.72**	0.0-11.0
Eos %	2.00±1.47	2.64±0.69	1.56±0.64	2.28±0.66	0.0-8.0
Bas %	0.42±0.11	0.44±0.15	0.52±0.16	0.66±0.13	0.0-2.5

WBC: White blood cells, RBC: Red blood cells, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, Neu: Neutrophils, Lym: Lymphocytes, Mon: Monocytes, Eos: Eosinophils, Bas: Basophils, Neu%: Neutrophils%, Lym%: Lymphocytes%, Mon%: Monocytes%, Eos%: Eosinophils%, Bas%: Basophils%
*: The value determined at 24-h in Alpine goats is significantly different ($p<0.05$) from 0-h.
**: The value determined at 24-h in Saanen goats is significantly different ($p<0.05$) from 0-h.

Table 2. Effects on biochemical parameters (mean ± SD) after oral administration of a single dose of robenacoxib (4 mg/kg) to Alpine and Saanen goats (n=5)

Biochemical Parameters	Alpine		Saanen		Reference Range
	0-h	24-h	0-h	24-h	
BUN (mg/dL)	16.26±1.41	20.10±1.69*	15.52±2.40	18.88±3.84	10-28
CRE (mg/dL)	0.46±0.03	0.44±0.04	0.43±0.06	0.39±0.06	0.3-0.8
TP (g/L)	69.78±2.76	68.34±2.44	72.82±4.57	72.62±5.44	65-75
Alb (g/L)	31.23±1.42	31.52±1.54	31.37±1.84	30.58±1.69	27-45
AST (U/L)	92.60±8.35	82.00±9.27*	78.60±9.63	88.80±14.07	66-230
ALT (U/L)	26.20±2.59	21.60±2.07*	19.00±5.39	19.40±4.51	15-52
GGT (U/L)	42.00±6.52	44.80±7.05*	45.80±9.04	47.80±8.11**	20-56
LDH (U/L)	400.20±48.54	356.60±53.80*	361.20±33.70	413.00±62.93**	0-400
CK (U/L)	184.60±20.19	173.60±41.29	192.80±16.27	217.40±48.60	116-464

BUN: Blood urea nitrogen, CRE: Creatinine, TP: Total protein, Alb: Albumin, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma glutamyltransferase, LDH: Lactate dehydrogenase, CK: Creatine kinase
*: The value determined at 24-h in Alpine goats is significantly different ($p<0.05$) from 0-h.
**: The value determined at 24-h in Saanen goats is significantly different ($p<0.05$) from 0-h.

DISCUSSION

NSAIDs are the group most often used in human and veterinary medicine. The use of NSAIDs off-label in painful and inflammatory conditions raises concern about their safety in sheep and goats because there are no approved NSAIDs for these species. Studies evaluating hematological and biochemical parameters after administration of NSAIDs with greater COX-2 selectivity than COX-1 to farm animals are limited (Durna Çorum and Yıldız, 2020), and no studies investigating the effects of COXIBs on ruminants were found in the literature review. This is the first study to evaluate hemato-

logical and biochemical parameters after oral administration of robenacoxib in different goat breeds. This study's results demonstrated no significant effects on hematological parameters in Alpine and Saanen goats after a 4 mg/kg single oral dose of robenacoxib at 24-h treatment. Similarly, there were no significant alterations in biochemical parameters in Alpine goats except for a significant increase in LDH enzyme activity at 24-h in Saanen goats.

The gastrointestinal tract, kidney, liver, and inhibition of blood clotting are the main targets of NSAID toxicity (Warner et al., 1999; Flower, 2003). Robenacoxib acts by inhibiting COX-2 specifically, which is responsible for prostaglandin synthesis, which is an essential component of pain. The selective COX-2 NSAID displayed several adverse effects regarding the integrity of hepatocellular damage, renal impairment, and raising WBC, with varying effects on different leukocyte subtypes (Wright, 2002). No important changes were determined in the hematological parameters after 24-h robenacoxib treatment in both goat breeds in this study. Furthermore, observed changes in hematological parameters for robenacoxib-treated Alpine goats were determined within their normal range. The decreases in RBC, HCT, and increases in MCH and Mon in both Alpine and Saanen goats were within the reference values, and similar results were obtained from celecoxib-treated healthy adults (Leese et al., 2000). Tabrizi et al. (2006) reported that celecoxib did not have significant effects on hematological results including WBC, RBC, and platelet counts, as well as the levels of PCV, HGB, MCV, MCH, and MCHC in dogs. Similar to these findings, meloxicam and ketoprofen did not alter the hematological parameters of healthy ponies (Pozzobon et al., 2009). Villegas et al. (2002) reported that piroxicam and meloxicam did not significantly affect hematological parameters in rats.

Some enzymes, proteins, and nucleic acids in the blood associated with specific tissue damage can be used to detect most drug-related adverse effects and, in some cases, to determine the prognosis (Tang and Lu, 2010; Özdemir and Traş, 2018). Measured in living beings, CRE and BUN are used to define kidney damage, AST, ALT, Alb, GGT, LDH, and TP are used to define liver damage, and CK is used to define heart damage (Gowda et al., 2009; Kim and Moon, 2012). Recently, it was reported that there was no evidence of robenacoxib toxicity in the liver or kidneys of cats or dogs. According to this research, in cats and dogs treated with robenacoxib subcutaneously and orally, alkaline phosphatase, ALT, AST, GGT, CRE, and BUN were not affected when compared to baseline levels or controls (Toutain et al., 2017; Heit et al., 2020). Previously, the critical role of COX-2 in maintaining kidney function was demonstrated by Gertz et al. (2002) and Whelton et al. (2000). Therefore, the adverse renal effects of COXIBs may be similar to those of non-selective COX inhibitors. In cases of acute kidney injury caused by NSAIDs, serum CRE and BUN values increase (Harris, 2006). There was no difference in serum CRE level between 0-h and 24-h in both goat breeds, while a statistically significant increased BUN value was found in Alpine goats at 24-h compared to 0-h within the reference range in the current study. Furthermore, lower values for AST and ALT can be detected in renal failure cases compared to the normal population (Ersoy, 2012). Similar to this finding, AST and ALT enzyme activities statistically decreased at 24-h after robenacoxib administration in Alpine goats compared to 0-h in the present study. The possible relevance of elevated serum GGT activity with the development of chronic kidney disease is known, but the literature on this subject is controversial and very limited in number (Noborisaka et al., 2013). In this study, a statistically increase in GGT activity at 24-h compared to 0-h was detected in both Alpine and Saanen goats. Firocoxib, one of the coxibs, had been administered orally at a dose of 5 mg/kg to healthy dogs, an increase in GGT activity had been determined within the reference range, but the researchers did not find it related to firocoxib (Steagall et al., 2007). In Saanen goats, an increase in LDH level over the reference range was observed at the 24-h. Laboratory abnormalities such as increased LDH is apparent in thrombotic microangiopathy. Thrombotic microangiopathy associated with pharmaceutical agents is usually caused by disorders of the hemolytic uremic syndrome (Shirali and Perazella, 2015). Low LDH is a rare condition and in this study, the decrease in LDH at 24-h in Alpine goats could not be related to robenacoxib treatment in the current study.

In this study, it was determined that robenacoxib administered orally at a dose of 4 mg/kg to different goat breeds did not cause any change in hematological and biochemical parameters except LDH enzyme activity in Saanen goats. These results show that oral robenacoxib administration does not cause any pathological changes in hematological and serum biochemical parameters in goats and appears to have been well tolerated by goats.

CONCLUSION

The results obtained from this study are the first data that the effects of oral administered robenacoxib on hematological and serum biochemical parameters in Alpine and Saanen goats. It was showed that statistical differences of hematological and biochemical parameters were within the normal limits in Alpine and Saanen goats except for LDH enzyme activity in Saanen goats. Moreover, while no differences were observed in hematological parameters according to goat breeds, when biochemical parameters are evaluated, it can be said that Alpine breed is exposed to adverse drug effects compared to Saanen breed. Administration of robenacoxib did not cause any adverse effects on hematological parameters or serum biochemisty in both goat breeds and it was suggested that it may be used as an alternative to existing NSAIDs. Furthermore, there is a need to investigate the effects of robenacoxib in high doses, long-term use, and disease conditions, especially on the kidney.

DECLARATIONS

Ethics Approval

All protocols in animals were approved by the Ethics Committee of the Cukurova University, Health Sciences Experimental Application and Research Center.

Conflict of Interest

The authors declare that they have no known competing fi-

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

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: ZOK

Data collection and analysis: ZOK, PPB

Drafting of the manuscript: ZOK, PPB

Critical review: ZOK

Data Availability

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

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Research Article

Prevalence of *Listeria monocytogenes* in dairy products in Turkey: A meta-analysis

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ABSTRACT

This study was aimed to conduct a meta-analysis to determine the prevalence of Listeria monocytogenes in dairy products in Turkey. A systematic literature search was conducted using the Medline/PubMed, Science Direct, Web of Science, and Scopus databases to identify studies reporting the prevalence of L. monocytogenes in dairy products. The search was conducted using the following keywords: ("ice cream" or "curd" or "cream" or "cheese" or "butter" or "yoghurt" or "dairy" or "milk") and ("Listeriosis" or "Listeria monocytogenes" or "Listeria") and ("Turkiye" or "Turkey") and ("seropositivity" or "seroprevalence" or "prevalence"). A comprehensive meta-analysis included a total of 4615 dairy products from the 26 studies. The overall molecular prevalence of L. monocytogenes in dairy products in Turkey was estimated to be 4.3% (95% confidence interval [CI]: 3.7-4.9%). The I2 value of 87.4% (CI95%82.8–90.8) suggested high heterogeneity, with a τ2 of -0.6227 (CI95%-4.6556–3.4102), and an X2 statistic of 199.0 ($P < 0.0001$). In conclusion, this meta-analysis reveals a significant prevalence of L. monocytogenes in dairy products in Turkey. The findings highlight the need for improved control measures to minimize the risk of contamination and ensure consumer safety. By implementing rigorous hygiene practices, enhancing monitoring systems, and promoting collaborative efforts between industry and regulatory bodies, the dairy sector in Turkey can effectively mitigate the risks associated with L. monocytogenes and maintain high standards of food safety.

INTRODUCTION

Listeriosis is a severe and significant food-borne disease caused by the bacterium *Listeria monocytogenes* (Ulusoy and Chirkena, 2019). This organism can contaminate food at various stages of production before it is consumed. Upon ingestion, the pathogen traverses the intestinal barrier and disseminates through the bloodstream and lymphatic system, ultimately reaching the liver and spleen, where it can proliferate (Andersson et al., 2015). *L. monocytogenes* is an opportunistic human pathogen that can cause meningitis or septicaemia, with a higher risk for pregnant women, the eldexxxxxxrly, and individuals with compromised immune systems. Immunocompromised individuals are particularly susceptible to this intracellular pathogen (Ramaswarmy et al., 2007). In pregnant women, *L. monocytogenes* can lead to severe infection in the fetus through vertical transmission via the placenta (Cossart, 2011). According to the World Health Organization report (2018), pregnant women are approximately 20 times more likely to contract *Listeriosis* compared to healthy adults, as the disease can result in miscarriage or stillbirth. Furthermore, individuals with HIV/AIDS are at least 300 times more susceptible to the infection compared to those with a normal immune system.

In Turkey, the dairy industry stands out as a highly dynamic sector within the farming industry (Akin et al., 2022). Turkey is recognized as the 10th largest milk producer globally, according to Yonar et al. (2022). As of 2021, Turkey's annual milk production amounts to approximately 23.2 million tonnes, with milking cows contributing nearly 21.4 million tonnes (Tuik, 2022). The collected milk undergoes processing to produce various dairy products, including drinking milk, cheese, yogurt, ayran, butter, kefir, milk cream, and ice cream (Bor, 2014). *L. monocytogenes* can contaminate a wide range of food products, including dairy items. As in other countries, Turkey also faces the potential risk of *L. monocytogenes* contamination in its dairy products (Cetinkaya and Soyutemiz, 2007; Kevenk and Gulel, 2016).

Meta-analysis is a statistical technique widely employed in the field of food science and nutrition to amalgamate and analyze data from multiple independent studies (Bouras et al., 2019). One primary advantage is that meta-analysis allows researchers to obtain a more precise estimate of the effect of a specific food or dietary pattern on health outcomes (Kelley and Kelley, 2019). By pooling data from multiple studies, meta-analysis enhances the statistical power of the analysis and mitigates the impact of random variation in individual studies, thereby yielding a more accurate estimation of the true effect size (Lee, 2019). Furthermore, meta-analysis facilitates the identification of sources of heterogeneity or inconsistency among studies, enabling exploration of potential biases or confounding factors. Consequently, meta-analysis provides a robust and comprehensive evaluation of the evidence base in food-related studies, informing future research directions and shaping public health policies (Nardi et al., 2020).

To the best of author knowledge, no previous meta-analysis

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has specifically focused on the prevalence of *L. monocytogenes* in dairy products in Turkey. Therefore, the aim of this study was to conduct a meta-analysis to determine the prevalence of *L. monocytogenes* in dairy products in Turkey and provide insights into the potential public health risks associated with the consumption of contaminated dairy products. Furthermore, the study has the potential to guide the implementation of effective preventive measures, ultimately reducing the risk of *L. monocytogenes* transmission to humans.

MATERIALS and METHODS

Literature search and study selection

The systematic review and meta-analysis adhered to the guidelines set forth by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al., 2009). A thorough literature search was conducted across multiple databases, including Medline/PubMed, Science Direct, Web of Science, and Scopus, to identify relevant studies reporting on the prevalence of *L. monocytogenes* in dairy samples. The search was conducted using the following keywords: ("ice cream" or "curd" or "cream" or "cheese" or "butter" or "yoğurt" or "dairy" or "milk") and ("Listeriosis" or "Listeria monocytogenes" or "Listeria") and ("Turkiye" or "Turkey") and ("seropositivity" or "seroprevalence" or "prevalence"). The search was confined to studies published in the English language within the timeframe of January 2000 to July 2023.

Inclusion criteria

In order to identify any additional pertinent studies, a manual search of the reference lists of the identified studies was conducted. The full texts of potentially eligible studies were carefully reviewed to assess their compliance with the inclusion criteria. To be considered eligible for inclusion, studies had to meet the following criteria: (1) report on the prevalence of *L. monocytogenes* in dairy samples, (2) provide adequate data for calculating the prevalence estimate and its confidence interval, and (3) be original studies rather than review articles.

Data extraction and meta-analysis

A standardized form was used to extract data from eligible studies. This form included the following information: author, year of publication, study area, period of study, kind of dairy product, number of dairy products tested, number of positive samples, prevalence, and confidence interval.

The meta-analysis conducted in this study utilized the prevalence of *L. monocytogenes* detected in dairy products as the dependent variable, serving as the effect size for the analysis. The methodology employed in this meta-analysis followed the previous approach outlined by Wang (2018). Initially, the heterogeneity among the included studies was assessed using Cochran's Q (X^2) test, which examines the null hypothesis of homogeneity. Additionally, the degree of heterogeneity was quantified using the Higgins' I^2 statistic proposed by Borenstein et al. (2021). Considering the observed high level of heterogeneity, a random-effects model was employed to estimate the overall weighted prevalence of *L. monocytogenes*. This model accounted for both within-study variance (sampling error) and between-studies variance (τ^2). The results of the meta-analysis, including the corresponding 95% confidence interval (CI)s, were presented using forest plots. To investigate the possibility of publication bias for studies with low or high effect sizes, Egger's test was utilized (Egger et al., 1997). All statistical analysis were performed using commercial software (Med-Calc; version 20.110; MedCalc Software Ltd).

RESULTS

After conducting an initial search, a total of 210 articles were identified. Among them, 26 articles were deemed suitable based on the inclusion criteria. The study selection process is outlined in Figure 1, while the characteristics of the selected

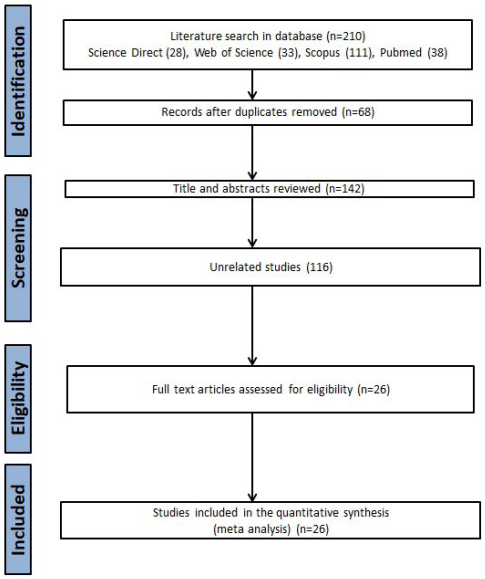

Figure 1. PRISMA flow diagram depicting the study selection process for the meta-analysis investigating the prevalence and source analysis of Listeria monocytogenes in dairy products.

studies can be found in Table 1. These studies were carried out in six distinct geographical regions of Turkey: the Marmara region (7 studies), Black Sea region (5 studies), Central Anatolian region (4 studies), Mediterranean region (3 studies), Eastern Anatolian region (1 study), and Southeastern Anatolian region (4 studies). Moreover, the geographical categorization of one study remained unspecified, while one study encompassed more than two distinct geographical regions. These studies were published between 2001 and 2020. A comprehensive meta-analysis included a total of 4615 dairy products from the 26 studies (Table 1).

The median size of eligible studies was 6, and out of the total 4615 samples, 231 were diagnosed as positive by serologic methods. The overall molecular prevalence of *L. monocytogenes* in dairy products in Turkey was estimated to be 4.3% (CI_{95}%: 3.7-4.9%). The I^2 value of 87.4% (CI_{95}%82.8–90.8) suggested high heterogeneity, with a τ^2 of -0.6227 (CI_{95}%-4.6556–3.4102), and an X^2 statistic of 199.0 ($P < 0.0001$). The overall meta-analysis is shown in a forest plot (Figure 2). The meta-analysis showed no significant evidence of publication bias based on Egger's test ($P = 0.7527$).

Table 1. Characteristics and main results of the eligible studies ordered by Prevalence of Listeria monocytogenes in dairy products in Turkey.

Author and Year	Geographical region	Period of study	Dairy product	Ns	Ps	Proportion (95% CI)
Kahraman et al. 2010	Marmara	2007-2008	Cheese	280	7	2.5 (1.0-5.1)
Kevenk and Gulel, 2016	Black Sea	2011-2012	Butter, cheese, cokelek, ice cream, kuymak	210	14	6.7 (3.7-10.9)
Kevenk and Koluman, 2022	Central Anatolian	2020-2021	Cheese	400	44	11.0 (8.1-14.5)
Karadal and Yildirim, 2014	Central Anatolian	2011	Cheese	200	2	1.0 (0.1-3.6)
Matyar et al. 2010	Mediterranean	2008	Cheese, ice cream, milk	101	8	7.9 (3.5-15.0)
Oktem et al. 2006	Central Anatolian	2001-2002	Cheese	100	4	4.0 (1.1-9.9)
Sagun et al. 2001	Eastern Anatolian	ND	Cheese, milk	504	13	2.6 (1.4-4.4)
Turhan, 2019	Mediterranean	ND	Cheese	20	0	0.0 (0.0-16.8)
Tasci et al. 2010	Mediterranean	2007-2008	Milk	175	1	0.6 (0.0-3.1)
Yigin et al. 2020	Southeastern Anatolian	ND	Cheese	103	6	5.8 (2.2-12.2)
Sanlibaba et al. 2018	Central Anatolian	2017	Cheese, milk	110	5	4.5 (1.5-10.3)
Abay et al. 2012	ND	2008-2009	Milk	150	0	0.0 (0.0-2.4)
Aksoy et al. 2018	Southeastern Anatolian	2012-2013	Butter, cheese, milk	300	22	7.3 (4.6-10.9)
Arslan and Ozdemir, 2008	Black Sea	ND	Cheese	142	13	9.2 (5.0-15.1)
Aygun and Pehlivanlar, 2006	Southeastern Anatolian	ND	Butter, cheese, milk, yoghurt	157	2	1.3 (0.2-4.5)
Arslan and Ozdemir, 2020	Black Sea	2016	Cheese, ice cream	67	0	0.0 (0.0-5.4)
Aydin et al. 2019	Marmara	ND	Milk	150	4	2.7 (0.7-6.7)
Cagri-Mehmetoglu et al. 2011	Marmara	2008-2010	Cheese, milk	24	0	0.0 (0.0-14.2)
Babacan, 2020	Black Sea	2013-2017	Cream cake, ice cream	128	16	12.5 (7.3-19.5)
Cetinkaya et al. 2014	Marmara	2009-2010	Butter, cheese, ice cream, milk, yoghurt	196	0	0.0 (0.0-1.9)
Cokal et al. 2012	Marmara	2010-2011	Cheese, dessert	200	8	4.0 (1.7-7.7)
Durmaz et al. 2015	Southeastern Anatolian	ND	Milk	140	3	2.1 (0.4-6.1)
Guner and Telli, 2011	Mediterranean, Eastern and Central Anatolian	ND	Cheese	120	34	28.3 (20.5-37.3)
Ekici et al. 2019	Marmara	ND	Cheese, cream	200	6	3.0 (1.1-6.4)
Gulel et al. 2020	Black Sea	2012-2013	Cheese, cream, milk	188	7	3.7 (1.5-7.5)
Colak et al. 2007	Marmara	2004-2005	Cheese	250	12	4.8 (2.5-8.2)
Total				4615	231	4.3 (3.7-4.9)

ND: Not determined, Ns: Number of samples, Ps: Positive samples

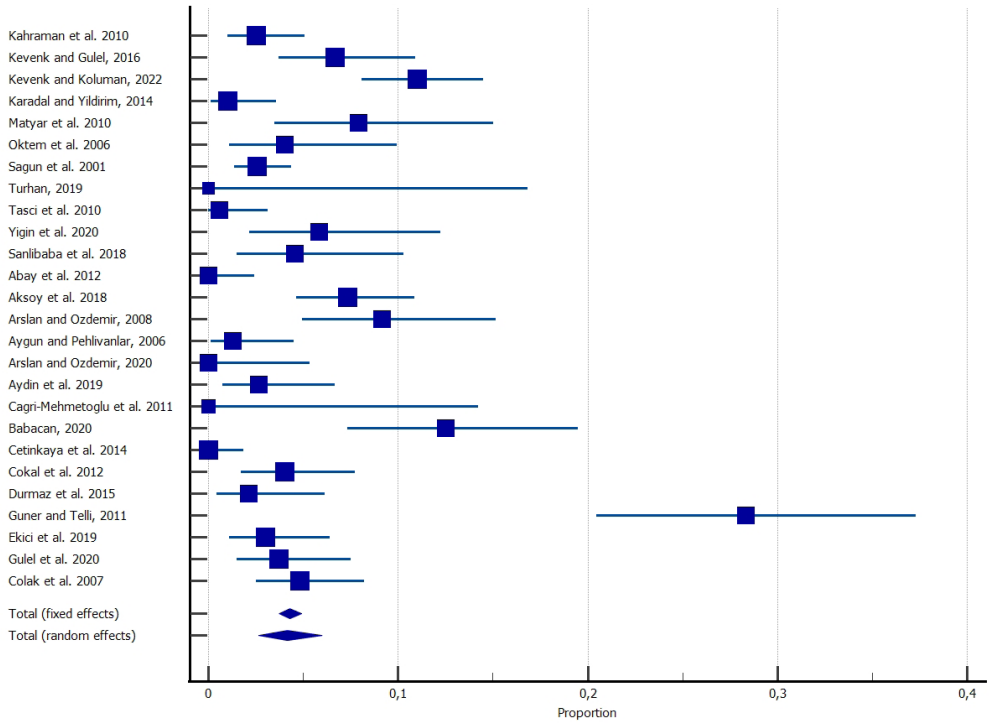

Figure 2. Forest plot illustrating the results of the meta-analysis evaluating the prevalence of Listeria monocytogenes in dairy products. The plot includes data from twenty-seven studies that met the inclusion criteria outlined in the meta-analysis.

DISCUSSION

The present study aimed to investigate the prevalence of *L. monocytogenes* in dairy products in Turkey through a comprehensive meta-analysis. The findings provide valuable insights into the prevalence and potential risks associated with *L. monocytogenes* contamination in the dairy industry, highlighting the need for effective control measures. The meta-analysis revealed an overall prevalence rate of 4.3% (CI_{95}% 3.7-4.9) across the included studies. This indicates a significant presence of *L. monocytogenes* in dairy products in Turkey. The high prevalence rate emphasizes the importance of implementing stringent quality control measures throughout the dairy production chain to minimize the risk of contamination.

Several factors contribute to the observed prevalence rates of *L. monocytogenes* in dairy products. One crucial factor is the contamination of raw milk, which serves as the primary source of *L. monocytogenes* ocytogenes in dairy production. Studies have shown that *L. monocytogenes* can colonize the udder and persist in raw milk, leading to subsequent contamination of dairy products during processing and packaging (Jiang et al., 2022). Inadequate hygiene practices during various stages of dairy production can also facilitate *L. monocytogenes* transmission. Contaminated equipment, unclean surfaces, and improper temperature control are all potential factors contributing to the survival and growth of *L. monocytogenes* in the production environment (Mazaheri et al., 2021).

The diversity of dairy products included in this meta-analysis is worth noting, as it reflects the wide range of products consumed in Turkey. The analysis encompassed milk, cheese, yogurt, and butter, among others. Interestingly, our findings indicate that certain types of dairy products especially cheese had high prevalence rate. This finding is consistent with previous studies that have identified cheese as a potential source of *L. monocytogenes* contamination due to its favorable pH and moisture conditions for bacterial growth (Falardeau et al., 2021).

Despite the valuable insights gained from this meta-analysis, several limitations should be acknowledged. The included studies varied in sample size, geographic location, and detection methods, which may introduce heterogeneity into the analysis. Publication bias is another potential limitation, as studies reporting higher prevalence rates may be more likely to be published. Future research should focus on standardizing sampling methods, laboratory techniques, and reporting criteria to enhance the comparability of data.

To mitigate the risks associated with *L. monocytogenes* in dairy products, a multifaceted approach involving industry and regulatory bodies is necessary. Dairy producers should prioritize regular monitoring of their production facilities, implement strict hygiene practices, and invest in advanced technologies for rapid detection and elimination of the pathogen (Ntuli et al., 2023). Additionally, regulatory authorities should enforce stringent food safety regulations, conduct regular inspections, and provide educational programs to raise awareness among dairy industry professionals about the importance of *L. monocytogenes* control (Ricci et al., 2018).

CONCLUSION

In conclusion, this meta-analysis reveals a significant prevalence of *L. monocytogenes* in dairy products in Turkey. The findings highlight the need for improved control measures to minimize the risk of contamination and ensure consumer safety. By implementing rigorous hygiene practices, enhancing monitoring systems, and promoting collaborative efforts between industry and regulatory bodies, the dairy sector in Turkey can effectively mitigate the risks associated with *L. monocytogenes* and maintain high standards of food safety.

DECLARATIONS

Ethics Approval

Not applicable.

Conflict of Interest

The authors declare no coflict of interest

Author contribution

Idea, concept and design: BY

Data collection and analysis: BY

Drafting of the manuscript: BY

Critical review: BY

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Case Report

Unusual eosinophilic gastritis with endoscopic examination in a cat

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ABSTRACT

Eosinophilic gastritis is a rare type of gastritis seen in cats and dogs, and it is still not completely understood. The patient was an 8-year-old Persian cat presenting with increasingly chronic vomiting. An endoscopic visualization of the stomach was performed after a physical examination, blood tests and imaging. During the endoscopic examination of the stomach, nodular pseudo-polyps diffusely disturbed in the antrum region and protruding toward the lumen were macroscopically observed. After examining the biopsy samples taken from these structures, the patient was diagnosed with eosinophilic gastritis.

INTRODUCTION

In cats and dogs, as in other mammals, the inflammatory disease of the stomach in its simplest form is called gastritis. It is classified as acute or chronic depending on the occurrence of inflammation. Gastritis is usually clinically associated with vomiting, dehydration, and metabolic acidosis. Hemorrhage, edema, excessive mucus secretion, abscesses, granulomas, foreign bodies or their penetration, parasites, various inflammatory reactions, erosions, and ulcers cause inflammatory reactions on the gastric mucosal surface. The etiology of eosinophilic gastritis is not fully understood. It is characterized by a diffuse eosinophilic infiltration in the distal part of the stomach (Neiger, 2010). Although eosinophilic gastritis is uncommon in domestic animals, there have been some cases in pet carnivores. Eosinophilic gastritis can be classified into three headings. In the first type, ingestion of parasite larvae may result in the occurrence of polyp-like hyperplasia in the stomach and intestinal epithelium, which sometimes cause pyloric obstruction. Hypersensitivity reactions can be observed in a second type in which eosinophils spread more diffusely by unknown trigger antigens. Peripheral eosinophilia occurs mostly in this type. Eosinophilic infiltration, which presents itself, especially in the small intestine, is identified as eosinophilic gastroenteritis. The third type is scirrhous eosinophilic gastritis of unknown causes in cats and dogs. Microscopically, lesions in eosinophilic gastritis appear as large areas of eosinophil infiltration in the mucosa and submucosa (Gelberg, 2017). Eosinophilic gastritis is usually reactions caused by food allergens. Although mucosal thickening is sometimes identified during ultrasound examination, clinical findings are not pathognomonic. Endoscopic biopsy applications can be advantageous in terms of duration of the procedure and damage to the tissue when compared to biopsy samples taken by laparoscopic methods. However, biopsies taken with endoscopic methods will remain superficial and may not provide information about mucosal lesions. The purpose of this study is to examine pseudopolyps found in the stomach cytologically and histopathologically.

MATERIALS and METHODS

An 8 year old, castrated, male Persian cat with increasing complaints of chronic vomiting was admitted to an animal hospital. According to its clinical history, it was an indoor cat, and it was fully vaccinated and had completed anti-parasitic treatments. The initial vomiting frequency was once or twice a month but eventually it began to occur every day. The patient had no complaints other than vomiting.

Blood, serum biochemistry, urine and hormone test conducted after the physical examination did not reveal any abnormalities that could cause vomiting. Hematology results were (Idexx Procyte Dx Hematology Analyser) RBC 9 (6.54-12.20) M/µL, HCT 37.9 (30.3 - 52.3)%, hemoglobin 12.8 (9.8 - 16.2) g/dl, MCV 42.1 (35.9 - 53..1) fL, MCH 14.2 (11.8 - 17.3) pg, MCHC 33.8 (28.1-35.8) g/dL, reticulocytes 9.9 (3-50) K/µL, WBC 8.69 (2.87-17.02) K/µL, neutrophils 4.86 (1.48 - 10.29) K/µL, lymphocytes 2.5 (0.92 - 6.88) K/µL, monocytes 0.29 (0.05 - 0.67) Kµ/L, eosinophils 0.94 (0.17 - 1.57) K/µL, basophils 0.10 (0.01 - 0.26) K/µL, and plateletes 285 (151 - 600) K/µL. Blood chemistry test (Idexx Catalyst One Chemistry) results were as follows: glucose 84 (74 - 159) mg/

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dL, creatinine 1.2 (0.8 - 2.4) mg/dL, urea 15 (16 - 36) mg/dL, SDMA 9 (0 - 14) µg/dL, UPC < 0.08 (non-proteinuric), BUN: creatinine ratio 12, phosphorus 4.3 (3.1 - 7.5) mg/dL, calcium 9 (7.8 - 11.3) mg/dL, total protein 6.4 (5.7 - 8.9) g/dL, albumin 2.7 (2.2 - 4.0) g/dL, globulin 3.7 (2.8 - 5.1) g/dL, albumin: globulin ratio 0.7, ALT 57 (12 - 130) U/L, ALP 29 (14 - 111) U/L, GGT 0 (0 - 4 U/L), total bilirubin < 0.1 (0.0 - 0.9) mg/dL, cholesterol 96 (65 - 225) mg/dL tT4 1.2 (0.8 - 4.7) µg/dL. Feline pancreas-specific lipase enzyme was in the normal range (Idexx Snapshot Dx Analyzer). A urine sample collected via cystocentesis was tested with a urine strip (Idexx Vetlab UA) and gave the following results: color, pale yellow, sg: 1.043, pH:6.5, urine protein; negative; glucose, negative; ketones, negative; blood/hemoglobin 50 ery/µL; bilirubin, negative; and urobilinogen: 1 µg/dL. A blood smear was performed to evaluate eosinophils. Correlations between blood smear results and the blood count determined.

On sonographic examination, a slight thickening of the muscular layer of the small intestine and 0.3 - 1 cm diameter cystic structures were observed in the right and left kidneys.

The decision was made for endoscopic imaging due to the lack of results indicating any factor that would induce vomit-

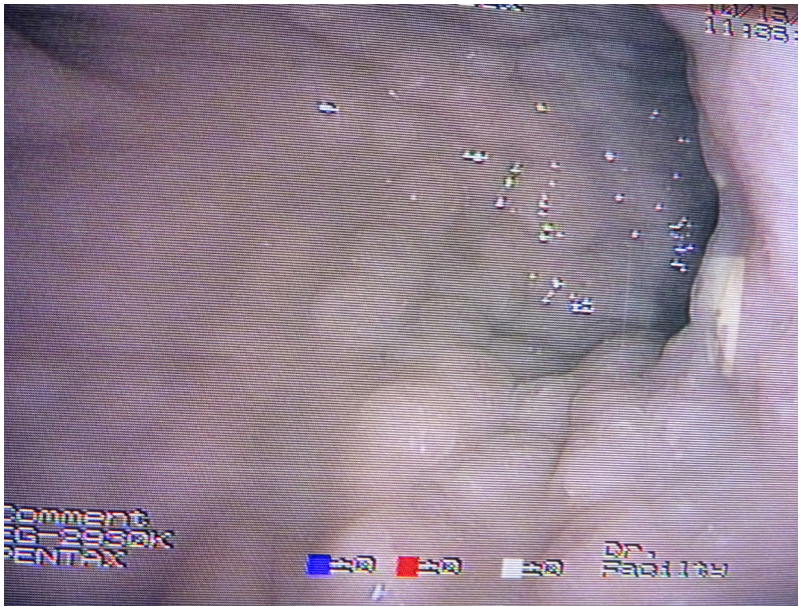

Figure 1. Diffusively distributed pseudo-polyps in the pyloric antrum. Biopsies were taken from this part.

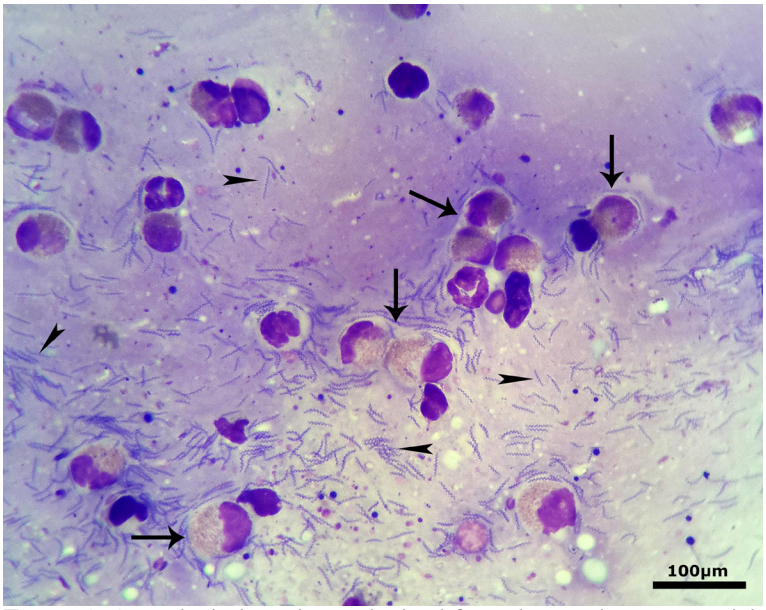

Figure 2. A cytological specimen obtained from the gastric antrum. High numbers of eosinophils (arrows) and spiral shaped bacteria (arrowheads) are shown (Wright's stain; bar 100 µm).

ing.

No macroscopic anomalies from the patient's mouth to the entrance of the stomach, including the esophagus, were found in the endoscopic examination with a video gastroscope (Pentax EG-2930K). Diffusely distributed pseudo polyps developing toward the lumen of the stomach were detected in the pyloric antrum (Figure 1). These foci were approximately 2 - 3 mm in size. Biopsies were taken from the structures in the pylorus region under endoscopic guidance. The biopsies were cytologically stained using Wright's staining method. Increased number of eosinophils was detected on a slightly eosinophilic background in cytologically examined samples under a light microscope (Figure 2). Eosinophil leukocytes were followed by neutrophil leukocytes and lymphocytes in terms of density. Spiral shaped bacteria become blue when stained with Wright's eosin methylene blue (Figure 2).

Biopsies were fixed in a 10% formalin solution and embedded in paraffin. 4 µm in thickness were stained with hematoxylin-eosin. Histopathological examination revealed a large amount of degranulated eosinophils in the lamina propria close to the lumen (Figure 3). According to the histopathological classification specified by the World Small Animal Veterinary Association (WSAVA) Gastrointestinal Standardization Group, the eosinophil leukocyte count should moderate, from 50 up to 100 at high magnification; 100 indicating a marked increase. In our sample, eosinophil leukocytes were markedly over 100 and scattered over wide areas of the lamina propria (Day et al. 2008). The number of eosinophil leukocytes in this area in the normal gastric mucosa is accepted to be between 0 and 2 at high magnification (Washabau et al. 2010). Mild atrophy was observed in the glands and extensive spiral-shaped bacteria were detected (Figure 3).

Vomiting ceased shortly after antibiotic (amoxicillin-clavulanate), antiemetic (maropitant) and H2 receptor antagonist (ranitidine) treatment had commenced. The diet switched to hydrolyzed protein containing food. Endoscopic examination of the stomach was not repeated due to the lack of further clinical symptoms.

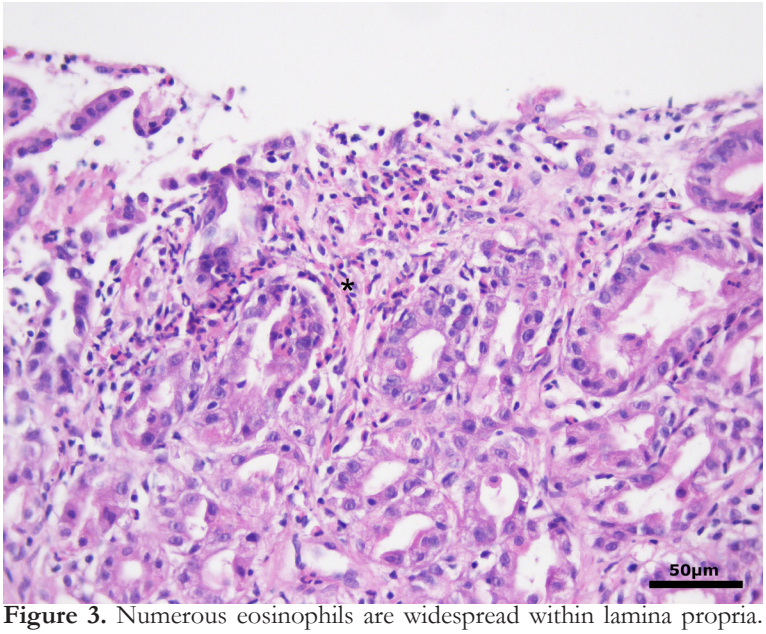

Figure 3. Numerous eosinophils are widespread within lamina propria. Mild atrophy is seen in the glands(asterisk) (Hematoxylin and eosin stain; 50 µm).

DISCUSSION

Eosinophilic gastritis is rare, and its etiology is not fully understood (Gelberg 2017; Votto et al. 2021). No laboratory findings and imaging methods, other than endoscopic imaging and biopsy examination, produce that lead to a diagnosis. Peripheral blood eosinophilia was found in approximately 80% of patients in human studies (Gonsalves, 2019). In our case we did not find a correlation between peripheral eosinophils and gastric inflammation. A diagnosis could only be reached by imaging the nodular pseudo polyps and evaluating the patient's general condition with histopathological and cytological examination of biopsies taken from the nodular pseudo polyps. Studies on eosinophilic gastritis in humans indicate two types of lesions: those showing nodular structure; or those erosional structures. The difference between nodular or ulcerative is morphological. Both types are variations of the same inflammatory response. However, some cases of eosinophilic gastritis do not conform to the two aforementioned types (Sato et al., 2017). A literature review failed to find mention of macroscopic imaging of pseudo polyps as nodules in veterinary medicine, even though it has previously been described in human medicine (Fisher et al. 2017; Sato et al. 2017). We believe that this type of imaging of gastric polyps we performed is a first in veterinary medicine. The ulceration of the diseased mucosa and flow of plasma proteins into the lumen in eosinophilic gastritis has been mentioned in veterinary medicine (Neiger, 2010).

Histopathological examination remains important despite the development of modern diagnostic methods (Rugge et al., 2020). The WSAVA has standardized it in order to ensure that gastric biopsies examined by pathologists are classified according to a certain standard. In this study, inflammation was interpreted as normal, mild, moderate, or severe depending on the number of leukocytes in a given area (Day et al., 2008). The high amount of eosinophilic leukocytes detected in the cytological examination of the endoscopic biopsy specimen in parallel with the histopathological examination may be sufficient for diagnosis. Since eosinophilic gastritis is usually limited to the mucosal layer (Neiger, 2010), endoscopic biopsy applications are thought to be sufficient in the diagnosis of eosinophilic gastritis without the need to take full-thickness biopsy samples. Thus, when taking the patient into consideration, anesthesia time, operation stress, and post-op care will not be necessary.

In previous human studies, some cases of gastritis were due to Helicobacter pylori causing an increase in the number of eosinophils in the stomach. In humans H. pylori is one of the most common causes of gastrointestinal diseases (Aydemir et al., 2004). In contrast to humans, in cats, H. heilmannii and H. felis are more frequently identified than H. pylori (Neiger et al., 1998). H. heilmanii can even be found in healthy cats' stomachs and may cause minimal inflammation (Norris et al., 1999). However, these species cannot be differentiated using only a light microscope (Neiger et al., 1998). Human-based studies have suggested that cobblestone nodules or polyps may be seen in eosinophilic gastritis (Connors, 2007).

We believe that a Helicobacter species caused the eosinophilic gastritis and the pseudo polyps in our case. Imaging and biopsy examinations of the stomach are recommended for long-term complaints that do not respond to symptomatic treatments. This type of gastritis might be more frequently encountered if there is an increase in the performance of endoscopic interventions on patients with such findings. Cytological examination of biopsy samples taken from lesions and mucosa will accelerate the diagnosis and, consequently, the treatment of some diseases.

DECLARATIONS

Ethics Approval

Not applicable.

Conflict of Interest

The authors declare that they have no competing interests

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: MFB, EYT

Data collection and analysis: MFB, EYT

Drafting of the manuscript: EYT,MFB

Critical review: MFB, EYT

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