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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).

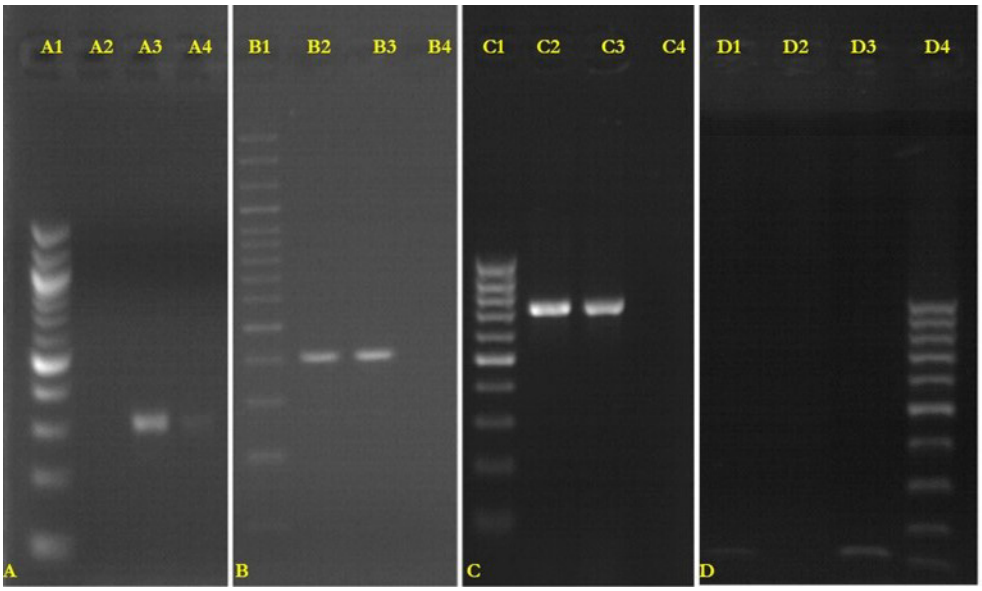

Figure 1. 1.5% agarose gel electrophoresis images of species-specific PCR products.
A: M. bovis specific PCR products (319 bp) A1: Clear Band DNA Marker 100 bp -Green (Eco Tech Biotechnology DM100) A2: Negative control A3: Positive control A4: Field strain
B: Erysipelothrix rhusiopathiae specific PCR 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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