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Original article

Prevalence of subclinical mastitis in sheep, etiological agents, and antimicrobial susceptibility in Northern Cyprus

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Abstract

In Northern Cyprus, around 51% of halloumi cheese is produced from sheep milk, and therefore the livelihood of the farmers mostly depends on the sheep milk production. However mastitis, an inflammation of the udder, significantly affects this production. Due to a lack of sufficient data concerning the prevalence, etiology, and antimicrobial resistance of sheep mastitis, there remains no effective method to control the disease. This study aims to estimate the prevalence of subclinical mastitis (SCM) and identify bacterial etiological agents and the antimicrobial susceptibility profiles of bacterial isolates in sheep in Northern Cyprus. A total of 227 milk samples taken from sheep were analysed using somatic cell count (SCC), bacteriological isolation-identification, and antimicrobial susceptibility procedures. Pathogens were isolated in 62 (27.3%) sheep milk samples. Somatic cell counts of more than 500000 cells/ml were found in 56 (24.6%). *S. aureus* (12.8%) was the most common isolate from the milk samples, followed by NAS (non-Aureus staphylococci) species (11.9%), *Escherichia coli* (0.9%), *Streptococci* (0.4%), *Bacillus spp*. (0.9%) and *Staph spp*. (0.4%). While a high resistance to sulphamethaxazole/trimetoprim (81.5%) was found, no resistance to gentamicin (10.6%) was found. The study findings indicate that subclinical mastitis is a serious problem in Cyprus. Therefore, continuous observation of subclinical mastitis and application of antibiogram tests to combat mastitis and antibiotic resistance and reduce economic losses are needed.

Keywords: antimicrobial susceptibility, Cyprus, *non-aureus staphylococci*, subclinic mastitis, *staphylococcus aureus*

Introduction

Northern Cyprus is characterised by its sheep and goat production, with livestock farming being a significant part of the local economy. According to a recent study conducted in 2023, the number of sheep contributing greatly to the economy in northern Cyprus totalled around 273,000. Northen Cyprus is also known for its unique geographical indications of the Turkish Cypriot halloumi cheese, which is made of both sheep and goat's milk. However, although mastitis disease negatively affects sheep milk and halloumi production on the island, no studies have been published on the causes or prevalence of mastitis. Additionally, the current concerns regarding mastitis in sheep are threatening public health (Gelasakis et al. 2015, Alba et al. 2019).

Mastitis is defined as an inflammation of the mammary gland (Al-Majali and Jawabeh 2003, Fragkou et al. 2014). While clinical mastitis cases in sheep show signs of severe inflammation in the udder and milk, no visible changes occur in subclinical mastitis. In studies, the prevalence of subclinical mastitis was estimated to be 7-92% worldwide (Ergun et al. 2009), and it was determined to be 0-9% in Cyprus (Jones 1984). Prevalence rates may vary depending on region, diagnostic test performed, race, and management. Mastitis control programs can also differ among countries due to specific factors, such as differences in the prevalence of pathogens, management and environmental factors. For this reason, pathogens causing mastitis and their prevalence should be well known to minimise losses due to mastitis. Mavrogenis et al. found that 1% of sheep udders were affected by clinical mastitis and that staphylococci were the most frequent cause of clinical mastitis (Mavrogenis et al. 1995). The most common bacteria isolated from cases of subclinical mastitis were nonaureus *Staphylococcus species*, *S. aureus, Streptococcus spp*., and *Enterobacteriaceae* (Ergun et al. 2009). Although the agents isolated in studies are often similar, the main agents vary between studies. While in some studies conducted on sheep with subclinical mastitis, NAS was shown to be the main etiological agent (Bergonier and Berthelot 2003, Gebrewahid et al. 2012, Queiroga 2017, Tvarozkova et al. 2021), in some of the others the main agent was determined to be *S. aureus* (Al-Majali and Jawabeh 2003, Hawari et al. 2014, Gelasakis et al. 2015, Vasileiou et al. 2018). Compared to clinical mastitis, subclinical mastitis is more difficult to diagnose due to the absence of clinical findings and causes higher economic losses (Queiroga 2017). Somatic cell counts (SCC), the California mastitis test, and bacterial culture are used to diagnose cases of subclinical mastitis in small ruminants (Fragkou et al. 2014).

The milk SCC value is used as an international standard tool in the evaluation of milk quality (Podhorecka et al. 2021). SCC in sheep and goats is much higher than in dairy cows. However, there are serious differences in many studies on the threshold values of SCC to distinguish healthy SCC from mastitis SCC in sheep. Studies for subclinical mastitis have defined threshold values ranging from 200 x 10^3 to > 1.0 x 10^6 cells/ml SCC (Bergonier and Berthelot 2003, Berthelot et al. 2006, Kiossis et al. 2007, Maurer and Schaeren 2007, Nunes et al. 2008, Fragkou et al. 2014). Some authors even set the limit for the diagnosis of subclinical mastitis as high as 1.5 x 10^6 cells/ml (Mavrogenis et al. 1995). There are continuing disagreements on the threshold values of SCC for the diagnosis of subclinical mastitis in sheep, where many factors such as the age of the sheep, lactation period, number of lactations, milk yield, and daily milking frequency are considered (Raynal-Ljutovac et al. 2007, Fragkou et al. 2014, Kaskous et al. 2022).

Mastitis is important for three reasons: sheep health, economic losses and human health. The main use of dairy sheep milk is traditionally in cheese processing. Control of mastitis and control of the hygienic quality of the milk are the main objectives in sheep breeding, producing milk and lambs (Bergonier and Berthelot 2003). While antimicrobials are an important tool in mastitis control programmes, the widespread and unsuitable use of antimicrobial agents in treatment and control programmes has led to antibiotic residue issues and the development of antibiotic-resistant bacterial species (Oliver and Murinda 2012, Paterna et al. 2014). Antimicrobial resistance is the most important problem that makes mastitis treatment difficult and causes failure. Studies on antibiotic resistance have shown that the resistance of microorganisms to antibiotics varies (Oliver and Murinda 2012). Although new generations of pharmacologically advanced antibiotics have been produced, the number of drug-resistant bacteria is still increasing. Additionally, recent research highlights the importance of bacterial resistance to antibiotics, and further studies are needed on this subject (Abdalhamed et al. 2018).

While the prevalence of mastitis and isolated pathogens varies by country and region, measures to prevent mastitis also vary regionally (Bradley 2002). Obtaining information regarding mastitis prevalence from a given region and the current pathogens is crucial in preventing mastitis and concomitant problems (Olivares-Pérez et al. 2015). In order to create specific and effective control methods, it is necessary to use the correct antibiotic based on the antibiogram results against the microorganisms that cause subclinical mastitis. The prevalence and aetiology of sheep mastitis

in Northern Cyprus as well as the antibiotic susceptibility of resident bacteria on islands are largely unknown, resulting in ineffective control programs. The aim of the present study includes (1) investigating the prevalence and aetiological agents of subclinical mastitis and (2) determining the antimicrobial susceptibility of bacterial agents isolated from sheep with subclinical mastitis in Northern Cyprus.

Materials and Methods

Study design and sample size

A cross-sectional study was conducted on two hundred Chios sheep randomly selected from four flocks in regions with the highest animal population in Northern Cyprus (T.R.N.C. Ministry of Agriculture and Natural Resources Veterinary Department Directorate 2023 Activity Report). The project was approved by the Near East University Animal Experimentation Local Ethics Committee with protocol number 2023/162.

Sampling of sheep and processing of samples

A total of 116 Chiros sheep with different parities and lactation stages were randomly selected and sampled from 10 commercial farms. The selected farms were considered as the reference population of the region. A cross sectional-random sampling study was conducted to assess the prevalence of subclinical mastitis in Chiros sheep. The sample size was determined according to the method used by Daniel (1999). The ewes were on pasture during the day and received concentrates at 200 g per day during milking. The sheep selected were clinically healthy, free from any signs of clinical mastitis and other palpable udder lesions. The sheep were milked by hand once a day. Preventive methods for bacterial transmission, such as teat dipping and dry-off treatment, were used by the milkers. A total of 227 milk samples from separate quarters were collected during lactation. The samples were taken during morning milking. Standard milk-sampling techniques were applied in the collecting, handling and storing of the samples. All of the milk samples were evaluated for SCC, bacteriological isolation-identification, and antibiotic susceptibility. For proper analyses, after 1-2 squirts of foremilk, 15 ml of milk samples were collected aseptically in sterile Falcon tubes (Isolab®, Germany) from all sheep. Before the foremilk was discarded, the teat was cleaned with 70% ethanol, and the samples placed into tubes were immediately transported to the laboratory (T.R.N.C. Ministry of Agriculture and Natural Resources, Veterinary Department, Animal Health Laboratory) in the cold (+4°C). Somatic cell counts were determined by using a flow cytometric method (CombiFoss 7, Hilleroed, Denmark). A SCC of $\geq$ 500 x 10^3 cell/ml milk was considered to be positive for subclinical mastitis (Tvarožková et al. 2020).

General and selective media were used for the isolation and identification of microorganisms from the ten microlitres of milk sample taken. Blood Agar (Merck), Edward's Medium (Oxoid), and MacConkey Agar (Merck) were used for the agent isolation, and the media were incubated for 24 hours under aerobic conditions at 37°C. Identification of the isolated microorganisms using an automated bacteria identification system was made according to their morphological, cultural, and biochemical characteristics (Quinn et al. 2002). Additionally, catalase test, coagulase test and mannitol fermentation test were used to differentiate *Staphylococcus aureus*. *Streptococcus* spp. was evaluated as Gram-positive bacteria that appear as a chain in the Gram staining result.

Antibiotic susceptibilities of the isolated bacteria were determined using the Kirby Bauer Disc Diffusion Method on Mueller-Hilton agar to 12 antibiotics (Bauer et al. 1966). For the antibiotic susceptibility tests, a total of 13 different antibiotic discs were used: amoxicillin/clavulanic acid (30 μg), enrofloxacin (5 μg), ceftiofur (30 μg), tilmicosin (15 μg), oxytetracycline (30 μg), gentamicin (10 μg), trimethoprim-sulfametaxazole (23.75/1.25 μg), lincomycin (15 μg), doxycycline (30 IU), florfenicol (30 μg), cephalexin (30 μg), amoxicillin (25 μg), and pencillin (10 units). The antibiogram test results were evaluated according to the criteria reported by the Novick JR and William J (1989).

Statistical analysis

The microbiological analysis, antibiotic resistance results of the milk samples included in the study, and the antibiotic resistance of each of the bacteria seen as a result of the microbiological analysis were calculated as "Frequency (N)- %". The descriptive statistics of the number of somatic cells were calculated and grouped as < 500.000 and $\geq$ 500.000. The distribution of the samples found to be negative or positive as a result of the microbiological analysis and the results of each bacteria in the somatic cell number groups were calculated.

Results

In the present study, 227 milk samples collected aseptically from the clinically healthy functioning mammary glands of 116 sheep were examined. 56 (24.6%) milk samples were collected, and somatic cell

Table 1. Relationship between bacterial culture and SCC[a] results in sheep milk samples.

			Bacterial Culture		Total
			Negative	Positive	
SCC cells/ml	Negative (<500 x 10^3)	n (%)	127 (74.3)	44 (25.7)	171
	Positive (>500 x 10^3)	n (%)	38 (67.9)	18 (32.1)	56
Total		n (%)	165 (72.7)	62 (27.3)	227

[a]SCCs of > 500.000 x 10^3 cell/ml milk were considered positive.

Table 2. Bacterial isolates from sheep milk samples (n:227) in sheep with subclinical mastitis.

Bacteria	n (%)
No growth	165 (72.7)
S. aureus	29 (12.8)
NAS	27 (11.9)
E. coli	2 (0.9)
Strep. spp	1 (0.4)
Bacil. spp	2 (0.9)
Staph. spp	1 (0.4)

Table 3. Relationship between SCC and bacterial cultures in subclinical mastitis.

			Bacteria							Total
			No growth	*S. aureus*	*CNS*	*E. coli*	*Strep. spp*	*Bacil. spp*	*Staph. spp*	
SCC	Negative (<500 x 10^3)	n (%)	127 (74.3)	24 (14.0)	16 (9.4)	0 (0)	1 (0.6)	2 (1.2)	1 (0.6)	171
	Positive (≥ 500 x 10^3)	n (%)	38 (67.9)	5 (8.9)	11 (19.6)	2 (3.6)	0 (0)	0 (0)	0 (0)	56
	Total	n (%)	165 (72.7)	29 (12.8)	27 (11.9)	2 (0.9)	1 (0.4)	2 (0.9)	1 (0.4)	227

counts greater than 500 × 10^3 cells/ml were found. SCC (n=227) ranged from 1000 to 34.921.000 cells/ml with an average SCC of 1.675.627 cells/ml.

Among the 227 milk samples subjected to bacteriological culture, the proportion of culture-positive samples was approximately 62 (27.3%). Of 56 (24.6%) SCC positive and the 62 bacteriologically positive milk samples, 18 were both SCC and bacteriologically positive (Table 1). However, 72.7% (165/227) of the samples showed no microbial growth. *S. aureus* (12.8%) was the most common isolate from milk samples, followed by NAS species (11.9%), *Escherichia coli* (0.9%), *Streptococcus spp.* (0.4%), *Bacillus spp*. (0.9%) and *Staph spp*. (0.4%). *S. aureus* was the most prevalent isolate from samples observing negative SCC results. The number of isolates of each bacterial species and respective percentages is shown in Table 2. The bacteria detected in the bacteriological cultures performed on SCC positive and negative sheep are shown in Table 3.

The antimicrobial susceptibility pattern of the milk samples is shown in Table 4. A higher Sulphamethaxazole/trimetroprim (81.5%) resistance was found, and no gentamicin resistance was found (10.6%) in all milk samples.

Discussion

In the study, the overall prevalence of subclinical mastitis in Chiros sheep was present 24.6%. These findings are in close agreement with those reported by Vasileiou et al. (2018) 26%, Gebrewahid et al. (2012)] 28.14%, Moawad and Osman (2005) 29.45%, and Ahmed et al. (1992) 25%. However, other studies have indicated a lower prevalence (McDougall et al. 2002, Al-Majali and Jawabeh 2005, Ergun et al. 2009,

Table 4. Antibiotic susceptibility in isolated agents.

	S. aureus n (%)	*CNS* n (%)	*Strep. spp*. n (%)	*E. coli* n (%)	*Bacillus* spp. n (%)	*Staph. Spp.* n (%)
Enrofloxacin	6 (20.7)	0	1(100.0)	0	0	1(100.0)
Ceftiofur	3 (10.3.)	1 (3.7)	1(100.0)	0	0	1(100.0)
Tilmicosin	1 (3.41)	1 (3.7)	0	0	0	1(100.0)
Oxytetracycline	3 (3.4)	1 (3.7)	0	0	0	0
Gentamicin	2 (6.9)	1 (3.7)	0	1 (50.0)	0	0
Sulphamethaxazole/trimetroprim	18 (62.1)	1 (3.7)	0	1 (50.0)	0	0
Lincomycin	1 (3.4)	0	0	0	0	0
Doxycycline	2 (6.92)	1 (3.7)	0	0	0	0
Florfenicol	0	2 (7.4)	0	0	0	0
Amoxycillin/clavulanic acid	5 (17.2)	6 (22.2)	1(100.0)	1 (50.0)	0	1(100.0)
Cephalexin	4 (13.8)	1 (3.7)	0	0	0	0
Amoxycillin	2 (6.9)	4 (14.8)	0	0	0	0
Pencillin g	0	2 (10.5)	0	0	0	0

Tvarožková et al. 2020) and a higher (Queiroga 2017) prevalence in sheep. In general, the incidence of subclinical mastitis in sheep varies from <10 to >50% (Bergonier and Berthelot 2003). There are no data on subclinical mastitis in sheep in Northern Cyprus. In a previous study conducted in Cyprus, the prevalence of subclinical mastitis was determined as 0-9%, and it was reported that this rate could reach 50% in some herds (Jones 1984). Mavrogenis et al. determined the incidence of clinical mastitis in Cyprus as 1% (Mavrogenis et al. 1995). In our study, the prevalence of mastitis was determined to be higher than previous studies conducted in Cyprus. The prevalence of subclinical mastitis may vary depending on the country, sheep breed, manegament, lactation period, season, and the type of tests or sampling methods (Al-Majali and Jawabeh 2003, Moawad and Osman 2005). In addition, the threshold values used, study design, and species differences should also be taken into account when comparing results between studies.

Currently, the most effective method for the detection of inflammation in the udder is to define increased cellular content in the milk. Since the milk SCC value is closely related to the health of the udder (Harmon 2001, Stocco et al. 2020), it is used as an international standard tool in the evaluation of milk quality (Podhorecka et al. 2021). Many studies conducted in a variety of different countries have presented various reference values for somatic cell numbers in sheep, but the threshold values for sheep somatic cell numbers are not as well defined as those of cows (Sevi et al. 1999, Riggio et al. 2013, Alekish et al. 2014). Additionally, many factors, such as the sheep's age, breed, lactation period, number of lactations, milk yield, sampling day and daily milking frequency affect the results of a somatic analysis. Consequently, these factors must be taken into consideration in any diagnosis of subclinical mastitis (Raynal-Ljutovac et al. 2007, Fragkou et al. 2014, Kaskous et al. 2022). There are considerable differences in SCC threshold values in sheep mastitis. Some studies (Berthelot et al. 2006, Fragkou et al. 2014) have suggested that values < 0.5 x 10^6 cells ml indicate a healthy mammary gland, while values > 1.0 x 10^6 cells ml indicate a mammary gland with subclinical mastitis. Some authors also considered 500 x 10^3 cells/ml as the physiological threshold of SCC (Bergonier and Berthelot 2003, Kiossis et al. 2007, Maurer and Schaeren 2007, Nunes et al. 2008). However, the data others proposed as 645 x 10^3 cells/ml was fever (Mavrogenis et al. 1995). Other workers (Swiderek et al. 2016) have used lower threshold values (200 x 10^3 cells/ml) when diagnosing subclinical mastitis. Leitner et al. derived a limit for indications of problems with udder health at 250 x 10^3 cells/ml (Leitner et al. 2008). Ozenc et al. (2011) detemined the limit for the diagnosis of subclinical mastitis to be 374 x 10^3 cells/ml. Kern et al. (2013) reported a similar threshold of SCC for detection of mastitis. Caboni et al. (2011) indicated a threshold of SCC as 265 x 10^3 cells/ml in sheep. In a study conducted in Cyprus, Mavrogenis et al. (1995) reported that the threshold level for subclinical mastitis in sheep was 1.5 x 10^6 cells/ml. However, this threshold value can be considered quite high compared to the presented study. There are disagreements in the literature regarding the SCC threshold values in the diagnosis of subclinical mastitis. Travazkova et al. (2020) evaluated SCC monthly and determined SCC as ≥500 × 10^3 in 92% of bacteria-positive milk samples. The same researchers reported that SCC ≥500 × 10^3 cells/ml is important for the detection of subclinical mastitis in sheep. In the pre-

sent study, in light of this information, the threshold value for the diagnosis of subclinical mastitis was selected as 500 x 10^3 cells/ml. Since a decision rule suggests that a mammary gland should be considered healthy (specificity = 75%) if each SCC is less than 0.500×10^6 cells/ml (Berthelot et al. 2006), we used the 500 x 10^3 cells/ml threshold value as a basis in our study. Hariharan et al. (2004) found that only 3.6% of the samples were positive for both bacteria and SCC. Although SCC was high in 39% of the samples, bacterial growth could not be detected. Similar to the previous study, the rate of SCC positive and bacteriology negative milk samples in this study was 67.9%. It is thought that the reason for not being able to isolate bacteria in a milk sample with positive SCC may be the presence of high SCC in milk after the elimination of infection or non-infectious mastitis. Additionally, in the presented study, bacteriological growth was detected in 25.3% of SCC negative samples. Consistent with Travazkova et al. (2020) the positive relationship between milk SCC and pathogen presence in sheep is not sufficiently clear. Staphylococcus spp., the most commonly isolated agent in cows, can be obtained from milk samples of cows without a significant increase in SCC (Petersson-Wolfe et al. 2010, Rall et al. 2014). Wald et al. (2019) reported that in low-SCC (<200.000 cells/mL) cows, 12 S. aureus and 32 CNS isolates were detected. As found in previous studies, it was concluded that determining a threshold value of less than 500 x 10^3 cells/ml for SCC in the diagnosis of subclinical mastitis in sheep would be more reliable for the diagnosis of subclinical mastitis.

In this study, bacterial growth was detected in 27.3% of milk samples obtained from sheep. Ergun et al. (2009) found a lower rate (6.4%) of bacteriological growth in milk samples compared to the present study. The bacterial growth rate in milk samples (36.73% obtained from sheep) was determined to be higher than the present study (Tvarozkova et al. 2021). Bacteria species and isolation rates from sheep milk with subclinical mastitis may vary depending on factors such as the breeding conditions of the animals, milking techniques and the hygiene and sanitation methods applied (Vasileiou et al. 2018, Tvarozkova et al. 2021). *S. aureus* was the most common bacteria detected in this study, representing a total percentage of 12.8% of positive cultures. Similar findings were reported in previous studies by Vasileiou et al. (2018), Al-Majali and Jawabreh (2003), Gelasakis et al. (2015), and Hawari et al. (2014). In another study, *S. aure*us was detected in 5.3% of positive bacteriological samples (Tvarožková et al. 2014). Moroni et al. (2007) isolated *S. aureus* in 8.4% of infected milk samples. In some regions, *S. aureus* was isolated from subclinical mastitis with a percentage of 6.2% (Bergonier and Berthelot 2003)-9.3% (Zigo et al. 2011). *S. aureus* spreads between animals via the milking machine and/or the milker's hands (Bergonier and Berthelot 2003). *Staphylococcus* species exhibit abilities as opportunistic behaviour in mammary gland infections when the host's immunity is weakened. *S. aureus* can produce heat-stable enterotoxin, and SCM is often undetectable. The use of infected milk for the production of halloumi may have a detrimental impact on public health. The high prevalence of this factor indicates poor hygiene during milking (Bradley 2002). In this study, similar to previous studies, the fact that *S. aureus* was the most frequently isolated pathogen in sheep milk samples shows a lack of appropriate hygiene standards in sheep enterprises in Cyprus, particularly during the milking process.

NAS were once thought to be harmless factors found in the normal flora of the skin; however, they are now known to be opportunistic pathogens causing mastitis. In our study, NAS was the second most frequently isolated agent (11.9%). However, several studies have found NAS to be the main etiological agent in sheep with subclinical mastitis (Bergonier and Berthelot 2003, Gebrewahid et al. 2012, Queiroga 2017, Tvarozkova et al. 2021). Because NAS is an environmental pathogen, it is generally isolated at a high rate. In previous studies, the incidence of NAS was found to be much higher, approximately 45% (Moroni et al. 2007, Gebrewahid et al. 2012). The incidence of NAS in our study was 11.9%, which is quite low compared to previous studies. Isolating NAS at different rates is reported to be important for studying pathogens. These differences in the isolation rates of coagulase--negative staphylococci in various studies may be due to differences in the methods used in identification and between geographical regions, as well as management applications.

The prevalence of *Streptococcus spp.* was 0.9% in our study, which is lower than the studies conducted previously in Egypt (50.4%) (El-Jakee et al. 2013), in Italy (9.5%) (Dore et al. 2016), and in Van province, Turkey (%6.79) (Gokhan and Gulaydin 2020). *Streptococcus spp*., an environmental bacterium, cannot be eliminated because they are found everywhere, but their spread is facilitated by poor hygiene practices. *Streptococcus spp.* isolates are generally known to cause sporadic mastitis cases due to hygiene deficiencies on the farm (Zdragas et al. 2005, Contreras and Rodríguez 2011). The lower incidence in Cyprus compared to other countries shows that hygiene conditions are better than in other countries.

Other bacteria causing subclinical mastitis were *E. coli, Bacil spp.* and *Staph spp.,* all of which have

been previously referred to as sheep subclinical mastitis pathogens and have been detected at different rates (Bergonier and Berthelot 2003, Hawari et al. 2014, Gelasakis et al. 2015). The less frequent isolation of these pathogens in the present study is consistent with previous studies.

Antimicrobial resistance is one of the most significant challenges in treating mastitis. Many studies have been conducted to determine the antibiotics to be used in the treatment of factors isolated from each mastitis case. Research has shown that the resistance of agents to antibiotics varies (Oliver and Murinda 2012). According to the antimicrobial susceptibility test results of *S. aureus*, a higher resistance was found against sulphamethaxazole/trimetroprim. However, it was found to be susceptible to gentamicin. In this study, NAS showed resistance to amoxicillin/clavulanic acid and had high sensitivity to amoxicillin. Both antibiotics are from the penicillin group of antibiotics. *E. coli* showed intermediate resistance to gentamicin (penicillin antibiotics), sulphamethaxazole/trimetroprim (sulfonamide antibiotic), amoxycillin/clavulanic acid (penicllin antibiotics), and high susceptibility to doxycycline (tetracycline antibiotics). In the present study, while *Strep spp.* showed high resistance to enrofloxacin (fluoroquinolones) and ceftiofur (Cephalosporins), high susceptibility to tilmicosin (macrolid antibiotics), oxytetracycline (tetracyclines), gentamicin, and pencillin G was evident. *Bacil spp.* were also susceptible to most of the antimicrobials. *Staph spp.* was highly resistant to most of the antimicrobials but was, however, susceptibelity to amoxycillin. Sulphamethaxazole/trimetroprim was found to be the most resistant antibiotic and gentamicin was found to be the most sensitive antibiotic in all milk samples. Some authors have reported increased resistance of *S. aureus* to streptomycin (48-87%) and penicillin (Lollai et al. 2008, Kunz et al. 2011). Naccari et al. determined that Stap spp. was susceptible to Tilmicosin (Leitner et al. 2008). Attili et al. (2016) determined *S. aureus* resistance to enrofloxacin. The greatest values of resistance of *S. aureus* were found for novobiocin 14.5%, erythromycin 12.8%, lincomycin 7.69%, and penicillin 7.69% (Vasıl' et al. 2018). Additionally, *NAS* has been reported to be resistant to gentamicin and thiamphenicol (Leitner et al. 2008). Higher levels of resistance were found for penicillin and tetracycline (Stocco et al. 2020). Holko et al. (2019) observed the highest resistance of NAS to lincomycin and neomycin. Ergun et al. (2009) found *Staphylococcus spp.* to be resistant to tetracycline, ampicillin, and penicillin; similar results were reported by Guler et al. (2005). Da Silva et al. (2004) found resistance in the *staphylococccus* species to penicillin, erythromycin, and trimethoprim-sulfamethoxazole, but and the *staphylococccus* species was susceptible to oxytetracycline. El-Jakee et al. (2013) found *streptecocci* to be susceptible to ampicillin, penicillin, and cefotoxime and resistant to vancomycin, tetracycline, and clindamycin. A study conducted in 2014 showed that *E. coli* were resistant to ampicillin, tetracycline, neomycin, sulfamethaxazole and gentamicin (Hawari et al. 2014). Data from previous studies are quite diverse and show similarities and differences. The fact that almost all bacteria showed resistance at rates of 70% or more to all antibiotics tested in the present study reflects, without making an antibiogram, the long-term and indiscriminate use of antibiotics in mastitis cases in Cyprus. Additionally, it is thought that the strain differences of the isolated factors may also play a role in the differences between studies. It is very important to perform antibiograms before the treatment of animals in order to increase the effectiveness of antibiotics and prevent the development of bacterial resistance.

Conclusion

This study observed and revealed the rate of sheep subclinical mastitis in Northern Cyprus, the bacterial profile isolated in milk samples and the antibiotic resistance of these factors. We can conclude that supporting somatic cell counts with microbiological methods and having an SCC threshold of $< 500 \times 10^3$ cells/ml for the diagnosis of subclinical mastitis are necessary for the successful management of mastitis. Subclinical mastitis is a major health problem in Northern Cyprus. Screening for subclinical mastitis at regular intervals, the isolation and identification of the agent, and the application of antibiogram tests are crucial in order to prevent mastitis and combat antibiotic resistance. The results of this study may also provide useful data for other studies concerning the aetiology of subclinical mastitis and antibiotic resistance status in Cyprus.

Additionally, both large and local breeders in Northern Cyprus must be informed about the prevalence of subclinical mastitis in the region and be encoureaged to use better milking hygiene habits to prevent economic loss and ensure healthier breeding habits.

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