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The Prevalance, Etiology and Antimicrobial Susceptibility of the Microorganisms in Subclinical Mastitis in Goats*

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Abstract: This study was performed to determine the prevalance, causative microorganisms and antimicrobial susceptibility of microorganisms in goat subclinical mastitis around Hatay. A total of 1010 mammary halves of 505 goats were examined by California Mastitis Test (CMT). The somatic cell counts (SCC) were determined by microscopic method. Isolation and identification of microorganisms were carried out by conventional microbiologic methods. *Staphylococci* were further differentiated by API-STAPH system. Antimicrobial susceptibility was determined by disc diffusion method. The prevalance of the subclinical mastitis was found 8.71 %. The most prevalent microorganism was *staphylococci* (71.5%). Microorganisms except *Staphylococci* were *Streptococci* (8%), *Bacillus spp.* (5.7%), *Escherischia coli* (4.5%), *Corynebacterium spp.* (3.4%), *Pseudomonas spp.* (2.3%) and *Acinetobacteri spp.* (2.3%). In addition, mix infection was defined in 2.3% of samples. Highly resistance was found against penicilin, erythromycin, oxytetracycline, gentamicin, amoxicillin. Slightly resistance was found against enrofloxacin, amoxicillin-clavulonic acid, kanamycin plus cephalexin and there was no resistance against cefalotin. It was concluded that, prevalance of subclinical mastitis should be cared; also *staphylococci* especially *Coagulase negative staphylococci* are the most commonly isolated bacteria in subclinical mastitis around Hatay. In goat mastitis diagnosis strong positive CMT results should be cared. CMT and SCC results should be supported with microbiologic tests.

Keywords: Diagnosis, Goat, Prevalence, Subclinic mastitis.

Keçilerde Subklinik Mastitislerde Prevalans, Etiyoloji ve Mikroorganizmaların Antibiyotik Duyarlılıkları

Öz: Çalışma, Hatay çevresinde keçilerde subklinik mastitis prevalansı, neden olan mikroorganizmalar ve mikroorganizmların antibiyotik duyarlıklıklarını belirlemek amacıyla düzenlendi. Beşyüzbeş keçiye ait 1010 meme lobu CMT ile muayene edildi. Somatik hücre sayısı (SHS) direkt mikroskopik yöntemle belirlendi. Mikroorganizmaların izolasyon ve identifikasyonu rutin mikrobiyolojik yöntemlerle gerçekleştirildi. Stafilokokların alt türlerinin belirlenmesi amacıyla API-STAPH system kullanıldı. Antibiyotik duyarlılık testlerinde disk difüzyon yöntemi kullanıldı. Subklinik mastitisin prevalansı %8.71 olarak belirlendi. En fazla izole edilen mikroorganizma %71.5 oranıyla Stafilokoklardı. Çalışmada *Stafilokoklar* dışında *Streptokoklar* (%8), *Basillus* spp. (%5.7), *E. coli* (%4.5), *Corynebacterium spp*. (%3.4), *Pseudomonas spp*. (%2.3) ve *Acinetobacteri spp*. (%2.3) de izole edildi. Ek olarak örneklerin % 2.3'ünde miks enfeksiyon saptandı. Antibiyotik duyarlılık testlerine göre penisilin, eritromisin, oksitetrasiklin, gentamisin, amoksisilin'e karşı yüksek oranda direç gözlendi. Enrofloksasin, amoksisilin klavulonik asit, kanamisin ve sefaleksin'e karşı orta düzeyde direnç saptanırken, sefalotine karşı direnç yoktu. Sonuç olarak Hatay çevresinde keçi sürülerinde subklinik mastitisin prevalansının dikkate değer seviyelerde olduğu, koagülaz negatif stafilokokların en fazla izole edilen mikroorganizmalar olduğu belirlendi. Keçilerde subklinik mastitis tanısında kuvvetli CMT reasksiyonlarının dikkate alınması gerektiği, CMT ve somatik hücre sayılarının mikrobiyolojik yöntemlerle desteklenmeleri gerektiği kanısına varıldı.

Anahtar Kelimeler: Keçi, Prevalans, Subklinik mastitis, Tanı.

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INTRODUCTION

Economically, sub-clinical mastitis is more important than clinical mastitis. It usually precedes the clinical form because of its longer duration also difficult to be detected it has adverse effects on milk quality and production also constitutes a reservoir of microorganisms that lead to infection of other animals within the herd (1-4). The prelevance of intramamillar infection in dairy goats is not only economic but also an hygienic and safety issue with respect to the bacteriological quality of milk in the dairy industry (5).

The prevalence of subclinical mastitis in small ruminants averages 6.5 and 40.2% (6-13). The causative etiologic agents of subclinical mastitis are mainly bacterial origin. Members of the genus *Staphylococci* are the main etiological agents involved in all forms of mastitis in goats. *Coagulase-negative staphylococci* (CNS) are the predominant etiologic agents in goats mastitis, especially in subclinical mastitis (14-17). Although CNS are less pathogenic than *S. aureus,* they can also produce persistent subclinical mastitis and even clinical mastitis (18,19), as well as producing thermostable enterotoxins (20-22). In the previous studies the bacteria isolated except the CNS were *Streptococci* (5,11,12), Bacillus spp. (12,13), *E. Coli* (5,11,12), *Corynebacterium spp.* (11), *Klepsiella spp. (*11), *Pseudomonas spp.* (5), *Micrococcus spp.* (13), *Candida and Yeast* (11).

Bacterial culture, somatic cell counts (SCC) and California mastitis test (CMT) are widely used to diagnose subclinical mastitis in small ruminants (9,23). SCC is the method based on counting of cells which take part in defense of mammary glands and used as the predictor of mammary health (9,10,23). CMT is the indirect and and subjective method of estimating the SCC based on scoring the degree of gel formation of milk with the CMT solution (9,24). The reagent used in CMT consists of the detergent sodium alkylarylsulfonate, sodium hydroxide and a pH indicator. The detergent lysis the somatic cells in milk releasing DNA in the solution of sodium hydroxide causing formation of a gel (24). Mc Dougall et al. (9) found a positive relationship between SCC and CMT therewithal reported that SCC and CMT are related to bacterial infection in goat milk. However, a positive relationship between SCC and mastitis in dairy goats remains controversial. Otherwise, Karzis et al. (25) stated that CMT and SCC were not alonely reliable methods the results should be supported with the bacterial culture. Contreras et al. (23) interprets the bacterial culture as the golden standart method for diagnosing mastitis in goats.

This study was performed to determine the prevalance of the subclinical mastitis, to identify the pathogens and, define the antibiotic susceptibility of the pathogens in goats.

MATERIALS and METHODS

This survey was carried out on 17 commercial dairy goat flocks on 1010 mammary halves of the 505 lactating clinically healthy dairy Damascus and Hair goats in Hatay. During the study local ethic rules were applied. The goats were milked handly once a day also the kids were suckled after milking. No preventive methods for bacterial transmission, such as antiseptics or gloves, were used during the milking process by the milkers. Teat dipping, neither lactation nor dry-off treatment was conducted in the herds before the study.

CMT solution used in cattle practice was also used in this study. The gradings of the CMT examinations were carried out according to Schalm et al. (26).

Milk samples were aseptically taken from separate udder halves and reached to the laboratory in 1 hour. SCC was determined by microscopic method. Ten µl of milk sample was spread on 5X20 mm area on a microscope slide and air dried. It was fixed by may grünwald solution for 5-7 minutes then stained by Giemsa solution (1 drop giemsa stock/1 ml distilled water) for 25 minutes. Twenty area was examined and the cells were counted. The average of the cell number was enumerated. Microscope

working factor was calculated. The SCC in 1 ml of the sample was found by the formula of Average cell numberXmicroscope working factorX100.

One hundred microliters of milk sample was spread on blood agar plates (supplemented with 5% defibrinated sheep blood). Aerobic incubation at 37 °C was performed and the plates were examined after 24 h. Identification of the colonies were done according to Gram staining, morphologic and hemolytic status. Tube coagulase test was applied to the Staphylococcus spp. colonies. The identification of the *staphylococci* were determined by API-STAPH tests.

The antibiotic susceptibility test was carried out by Kirby Bauer disc diffusion test using the following antibiotic discs; penicillin G (10U, Oxoid), amoxicillin alone (25 µg, Oxoid), gentamycin (10 µg, Oxoid), erythromycin (15 µg, Oxoid), oxytetracycline (30 µg, Oxoid), amoxicillin/clavulonic acid (30 µg, Oxoid), trimethoprim-sulphamethoxazole (25 µg, Oxoid), enrofloxacin (5 µg, Oxoid), cephalotine (30 µg, Oxoid,), kanamycine plus cefalexin, The susceptibility was calculated according to inhibition zone diameters. The inhibition zone diameters were evaluated according to the interpretive standards of Clinical Laboratory Standards Institude (27).

The samples both giving positive CMT reaction and microorganism isolation occured were mentioned to be mastitis.

RESULTS

212 milk samples belonging to 1010 mammary halves gave different grades of positive reactions to CMT (20.99%). Of these, microorganism isolation occured in 88 samples (41.5%). This ratio gave the prevalence of the mastitis as 8.71% of the total samples. The distribution of the 88 microorganism isolated samples according to CMT scores were; 34 samples CMT score 3, 41 samples CMT score 2, 9 samples were CMT score 1 and 4 samples trace. No microorganism isolation occured in CMT negative samples. Mean SCC of the samples were $832x10^3$, $834x10^3$, $1\,036x10^3$, $3\,900x10^3$ and $7\,932x\,10^3$ in the CMT negative, trace, CMT score 1, CMT score 2, CMT score 3 respectively. The CMT results and microorganism isolation rates were defined in table 1. The SCC and the microorganism isolation rates according to the CMT scores were summarized in table 2.

Table 1. The CMT results and microorganism isolation findings.
Tablo 1. CMT sonuçları ve mikroorganizma izolasyon bulguları.

Parameter	(n)	(%)
Samples	1010	100
CMT positive samples	212	20.99
Microbiologic isolation positive in total samples	88	8.71
Microorganism isolation in CMT negative samples	0	0
Microorganism isolation in CMT positive samples	88	41.5

CMT: California mastitis test

Table 2. The CMT scores, SCC and the microorganism isolation rates.
Tablo 2. CMT skorları, SCC ve mikroorganizma izolasyon oranları.

CMT score	Negative	Trace	1	2	3
SCC/ml	$832\ x10^3$	$834\ x10^3$	$1.036\ x10^3$	$3.900\ x10^3$	$7.932\ x10^3$
Microbiologic isolation	0	4	9	41	34
No Microbiologic isolation	All	70	47	5	2

CMT: California mastitis test, SCC: Somatic cell count

Most of the isolates (71.5%) were Staphylococci. Other bacteria isolated were Streptococci (7%), Bacillus spp. (5.7%), E.coli (4.5%), Corynebacterium spp. (3.4%), Pseudomonas spp.

(2.3%), and Acinetobacter (2.3%). The mix infection rate was 2.3%. The microorganisms isolated from the milk samples are shown in table 3.

Table 3. The microorganisms isolated from the milk samples.
Tablo 3. Süt örneklerinden izole edilen mikroorganizmalar.

Microorganisms	% (n)
Staphylococcus spp.	71.5 (63)
Streptococcus spp.	8 (7)
Bacillus	5.7 (5)
Escherichia coli	4.5 (4)
Corynobacterium spp.	3.4 (3)
Pseudomonas	2.3 (2)
Acinetobacter	2.3 (2)
Mix infection	2.3 (2)
Total	100 (88)

The majority of the staphylococcal isolate was S. intermedius (23.7%) a CPS. The CNS isolates were *S. capitis* (14.3%), *S. haemolyticus* (9.5%), *S. xylosis* (7.9%), *S. simulans* (7.9%), *S. caprae* (7.9%), *S. epidermidis* (6.4%), *S. warneii* (6.4%). Other species were *S. scuiri* (4.8%), *S. hominis* (3.2%), *S. auricularis* (3.2%). *Staphylococcus* species isolated from the milk samples in the study are demonstrated in table 4.

According to the antimicrobial susceptibility test reults of the staphylococci; a higher resistance was found against penicilin (77.7%), erythromycin (64.4%), oxytetracycline (53.3%), gentamicin (53.3%), amoxicillin alone (51.1%), the resistance against trimethoprim- sulfamethoxazole was 33.3 %. Slightly resistance was found against enrofloxacin (17.7%), amoxicillin-clavulanic acid (11.1%) and kanamycin plus cephalexin (0.6%) and no resistance was found against cefalotin (0%).

Table 4. *Staphylococcus* species identified from the milk samples.
Tablo 4. Süt örneklerinden identifiye edilen *Staphylococcus* türleri.

	Species	(n)	%
Coagulase positive	*S. aureus*	3	4.8
	S. intermedius	15	23.7
Coagulase negative	*S. capitis*	9	14.3
	S. haemolyticus	6	9.5
	S. xylosis	5	7.9
	S. simulans	5	7.9
	S. caprae	5	7.9
	S. epidermidis	4	6.4
	S. warneii	4	6.4
	S. sciuri	3	4.8
	S. hominis	2	3.2
	S. auricularis	2	3.2
	Total	63	100

S.: Staphylococcus

DISCUSSION and CONCLUSION

The prevalence of subclinical mastitis in small ruminants averages 6.5 and 40.2% (7-13). The prevalance of the subclinical mastitis is determined as 8.71% in this study. The range of the prevalance is among the data pointed out in the cited references.

Somatic cells are used as an index of milk quality for cow and goat milk. Milk somatic cell counts of goats are higher than milk somatic cell counts of cows and sheep (28). In goat milk, a sample can be classifed as mastitic if it has a SCC of $1x10^6$ cells per milliliter or greater (29). It is generally agreed in late lactation healthy goats often produce milk with more than one million somatic cells per ml (30-32). The CMT score has been shown to be positively associated with SCC with the probability of bacterial infection (33). Although Perrin et al. (34) reported that, CMT negative (scores 0 and 1) appeared to be more efficient than CMT positive (scores 2 and 3), which probably detects some false reactions which are not related to high SCC in goats, Contreras et al. (33), stated that the CMT Scores 2 and 3 discriminated between infected and uninfected udder glands. Haenlein (35), found that CMT levels in goat milk could determine the infected udder halves. Isolation of bacteria was associated with an

increased SCC, CMT and reduced impedance in both sheep and goats (9). Karzis et al. (25) reported that neither CMT nor SCC was sufficient alone in diagnosis of subclinical mastitis in dairy goats and the results should be approved by microbiological examinations. In the current study majority of the microorganism isolation provided in strong positive (+2 and +3) CMT samples. So, strong positive CMT results found to be predictive for microorganism isolation and mastitis determination, consistent with the findings of Contreras et al. (33). Nevertheless, negative CMT results and slightly positive reactions (trace and 1) should be evaluated carefully and these results should be confirmed by microbiologic examinations for the diagnosis of goat mastitis. Despite the fact that increased number of lactation, lower milk yield, increased number of parturition were reported to increase the SCC in goats without mastitis diagnosis (34,36), in the studies in which the number of parturition, lactation and milk yield was not evaluated and only one sample was taken the CMT results should be supported with the microbiological results as in this research.

Zeng et al. (32), reported that the microscopic method and the Fossomatic machine calibrated with goat milk standards gave comparable results of SCC in goat milk. Based on the CMT results, it was pointed out that the SCC of the samples counted by Fossomatic varied between $320x10^3$ and $730x10^3$, $2647x10^3$ and $6518x10^3$in the negative trace, 1, 2 and 3, respectively (29). Contreras et al. (33) determined that arithmetical means of SCC per microlitre for each CMT score were, 312×10^3 for Score 0 and traces; 1014×10^3 and Score 1; 2912×10^3 for Score 2; and 4950×10^3 for Score 3. In our study, the mean SCC results counted by direct microscopy was obtained 832×10^3, 834×10^3, 1.036×10^3, 3.900×10^3 and 7.932×10^3 in the negative, trace, 1, 2 and 3 scores of CMT, respectively.

The major types of bacteria involved in sheep and goats are *Staphylococci*, especially various coagulase-negative staphylococci (CNS), that are found on the skin of the udder and its surroundings (35,37-41). CNS was isolated between the ranges of 34.4% and 95.7%, (11,16,35,42,43). Consistent with the cited references, the major type of bacteria isolated in our study was staphylococci, at a rate of 71.5%, 68.2% of this was CNS. According to the API-STAPH results the CNS types isolated in the current study were*; S. capitis, S. haemolyticus, S. xylosis, S. simulans, S. caprae, S. epidermidis, S. warneii, S. sciuri, S. hominis, S. auricularis. S. intermedius coagulase* positive staphylococci were the most frequently isolated staphylococci (23.7) in the current study. *S. intermedius* was also isolated among the rates of 9.9 and 26.3% in various studies (22,42).

Da Silva et al. (17), found a resistance in staphylococccus species against penicillin at a rate of 29%, erythromycin and trimethoprim-sulfamethoxazole at a rate of 14%, and the staphylococci was susceptible to oxytetracycline at a rate of 100%. Aydın et al. (11), determined a higher resistance to penicilin, lactams and lactam inhibitors. Moroni et al. (10), reported that both Amoxicillin plus clavulanic acid and Amoxicillin alone showed a good efficacy against *CNS strains.* In the current study a higher resistance to Penicilin, erythromycin, oxytetracycline, gentamicin and amoxicillin alone was determined but a higher susceptibility was observed against amoxycillin plus clavulanic acid. Moroni et al. (10), stated that *w*hile cefoperazone showed poor activity against CNS strains, cephalonium showed good activity against *CNS strains*. Our results showed that staphylococcus species in the study showed a very high susceptibility against cephalosporins and their combinations such as kanamycin-cephalexim. Aydın et al. (11), showed that staphylococcal species were very susceptible to fluoroquinolone antibiotics as found in this research.

The bacteria isolated except the *staphylococci* were *Streptococci* (5,11,12), *Bacillus spp.*(12,13), *E. Coli* (5,11,12), *Corynobacterium spp.* (11), *Klepsiella spp.* (11)*, Pseudomonas spp.* (5), *Micrococcus spp.* (13), *Candida and Yeast* (11). Other bacteria isolated in this study were Streptococci, Bacillus spp., E.coli,

Corynebacterium spp., Pseudomonas spp., and Acinetobacter spp.

It was concluded that, staphylococci especially the CNS species are the agents most commonly isolated bacteria in subclinical mastitis in the goats herds of Hatay. In the diagnosis of goat mastitis especially the strong positive CMT results should be cared. CMT and SCC results should be supported with microbiologic tests. Also, the differences observed in antimicrobial susceptibility tests, defines the importance of antimicrobial susceptibility testing.

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