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Detection of Causative Agents in Goat Mastitis and their Antibiotic Resistance in Hatay Region

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SUMMARY Goat farming in many countries has significant economic importance. In recent years, there is a trend to goat products. Milk quality is associated with somatic cell counts that can be affected intra-mammary infections. The aim of this study is isolation and identification of causative bacterial, yeast and fungal mastitis agents in goat mastitis and detection of antibiotic resistance in bacterial isolates. Totally 220 milk samples from 110 goats in 11 farms were studied for isolation and identification of the agents. And antibacterial resistance of isolated microorganism were studied by disk diffusion method. Totally 30 strains isolated from samples and identification, isolation was made from 28 (12.73%) of 220 milk samples. Among these 30 isolates, 15 (50%) *Coagulase Negative Staphylococci*, 8 (26.67%) *S. aureus*, 5 (16.67%) *S. uberis* and 2 (6.67%) *E. coli* was identified. Identification was confirmed by molecular analyses from the direct culture of isolates. The highest resistance were found against to penicillin 21 (70%) and amoxicillin 19 (63.33%), and isolates highly sensitive to gentamicin 30 (100%), enrofloxacin 28 (93.3%), oxytetracycline 28 (93.3%) and amoxicillin + clavulanic acid 27 (90%). Periodical studies and constant monitoring of antibiotic resistance might be beneficial for therapy and prevention strategies and useful management acquisition of antibiotic resistance.

Key Words: *Antibiotic resistance, Goat milk, Mastitis*

ÖZET

Hatay Bölgesinde Keçi Mastitis Etkenlerinin ve Etkenlerin Antibiyotik Dirençlerinin Belirlenmesi

Birçok ülkede keçi yetiştiriciliği belirli bir ekonomik öneme sahiptir. Son yıllarda keçi ürünlerine yönelik bir eğilim vardır. Süt kalitesi meme içi infeksiyonlar tarafından etkilenen somatik hücre sayısı ile ilişkilidir. Bu çalışmanın amacı, keçilerde mastitise neden olan bakteri, maya ve mantarların izolasyonu ve identifikasyonu ve ayrıca izole edilen bakterilerin antibiyotik duyarlılıklarının belirlenmesidir. Bu amaçla, 11 farklı keçi çiftliğinde 110 keçiden alınan toplam 220 süt örneğinde etken izolasyonu ve identifikasyonu yapıldı. Ayrıca izole edilen bakteriyel izolatların antibiyotik dirençleri disk diffüzyon testi ile araştırıldı. Çalışmada toplanan 220 adet süt örneğinin 28 (%12.73)'inden 30 adet bakteri izole edildi. Bu 30 izolatın, 15 (%50)'i koagülaz negatif stafilokok, 8 (%26.67)'i *S. aureus*, 5 (%16.67)'i, *S. uberis* ve 2 (%6.67)'si de *E. coli* olarak identifiye edildi. İdentifikasyonlar, izolatların direkt kültürlerinden yapılan moleküler analizler ile teyit edildi. Bu suşlarda en yüksek antibiyotik direnci penisilin 21 (%70) ve amoksisilin 19 (%63.33)'e karşı bulundu, buna karşın izolatların 30 (%100)'u gentamisin'e, 28 (%93.3)'i enrofloksasin'e, 28 (%93.3)'i oksitetrasiklin'e ve 27 (%90)'si de amoksisilin + klavulanik asit'e duyarlı bulundu. Yapılacak periyodik çalışmalarla keçi mastitislerinden etkenlerin ve antibiyotik direnç durumlarının belirlenmesi hastalığın tedavi ve korunmasında oldukça yararlı veriler sağlayabilecektir.

Anahtar Kelimeler: *Antibiytik direnci, Keçi sütü, Mastitis*

INTRODUCTION

Goat farming has significant economic importance in many countries. In recent years, there has been a trend toward goat products. In recent times, goat-related products, such as goat's milk and goat's cheese, have become popular (Boyazoglu et al. 2005). There are about 500.000 goat farms in Turkey, and goat farming contributes to the incomes of 3.000.000 people (Dellal and Dellal 2005). Recently, it was emphasized that the rapid decreasing in

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the goats presence in Turkey due to insufficient macroeconomic policies, although economic and geographical conditions was very suitable for goat breeding. And, it was suggested that supporting and increasing of effective goat breeding (Gunlu and Alasahan 2010). The number of goats in Hatay province, while it was reported as 57.568 in the 2002, it was reported 142.185 in the 2014 year (Anon. 2016).

Milk quality is associated with somatic cell counts (SCCs), which are used as a marker of milk quality payments in many European Countries (Bergonier et al. 2003). Various factors can affect the SCC of goat milk, the breed and age of the animals, lactation stage, oestrus, milk production, management conditions and intra-mammary infections (mastitis) (Poutrel et al. 1997). Therefore, goat mastitis has implications for the economy and public health (Bergonier et al. 2003).

Staphylococcus spp., particularly *Staphylococcus aureus*, are the most commonly reported bacteria in goat mastitis (Bergonier et al. 2003). Many studies have reported that *S. aureus* can cause gangrenous mastitis when *Clostridium* spp. are also present (Islam et al. 2011). Besides *Staphyloccus* spp., *Streptococcus* spp. (*S. agalactiae*, *S. uberis* and *S. dysgalactiae*), *Pasteurella* (*Manhaemia*) *haemolytica*, *Corynebacterium pseudotuberculosis*, *Mycoplasma* spp. and rarely, *Salmonella* spp. and *Listeria* spp. have been reported to cause mastitis (Bergonier et al. 2003).

Studies about goat mastitis are very rare in Turkey (Ciftci et al. 1996; Aydin et al. 2009; Dogruer et al. 2010; Ilhan et al. 2011). The detection of causative agents in goat mastitis and their susceptibility to antibiotics can assist in both goat health and public health by initiating treatment of the disease or culling infected animals. The aim of this study was to detect causative agents of mastitis and the susceptibility of these agents to antibiotics in purebred and half-bred Damascus goats in the Hatay region.

MATERIALS and METHODS

Sampling

Consist of purebred and half-bred Damascus goats farms with an average 100-500 goats per farm were selected prior to sampling in Hatay Region, Turkey. The sampled farms were randomly selected from studied provinces. Four provinces of Hatay were visited and milk samples were collected from 4 farms in Yayladagı, 2 farms in Hassa, 1 farm in Reyhanli and 4 farms in Altinozu. Ten animals were selected randomly and both udder halves sampled without California Mastitis Test (CMT). Clinically infected goats were not selected for sampling in this study. Goats were sampled before the evening or morning milking. Udder halves were cleaned and disinfected prior to sampling with 70% alcohol and dried with sterile cotton. The first 3 squirts of milk were discarded and approximately 5-10 mL of milk samples was taken in sterile tubes for microbiological examinations. Samples were collected aseptically according to a standard procedure (IDF, 1985) and transferred to the laboratory within 1-3 h in a 4-8 °C cooler. Totally 220 milk samples were collected.

Microbiological Culture

The milk samples were mixed and 100 µl of milk were streaked onto Blood Agar and Edward's Medium (supplemented with 7% defibrinated sheep blood) and Mac Conkey's Lactose Agar and Sabouraud Dextrose Agar. Bacteriological and mycological isolation and identification were performed by the classical culture method and standard biochemical tests. For the bacterial identification, after incubation for 24 and 48 hours at 37°C, colonies in Blood Agar Plates were examined for colony characteristics, morphology and haemolysis properties. Sub-cultured pure colonies have (someone) undergo Gram staining, catalase test and oxidase test. And other biochemical tests; clumping factor, tube coagulase test, thermostable nuclease test, mannitol fermentation, the Christie–Atkins– Munch-Petersen (CAMP) reaction, esculin hydrolysis on Edwards Medium, sodium hippurate hydrolysis, nitrate reduction, gelatin hydrolyzation, urease production, Oxidation-Fermentation, motility in semi-solid medium, growing Mac Conkey Agar and Lactose fermentation test, IMVIC tests were carried out to identification of the isolates (Carter 1990; Quinn et al. 1994).

For the *Mycoplasma* spp. isolation, 1 ml of milk sample was transferred to 9 ml of PPLO broth medium (supplemented with horse sera, thallium acetate, and penicillin) and incubated at 37°C for two weeks under microaerophilic conditions. After the incubation, 100µl aliquots were transferred from the PPLO broth medium to PPLO agar (supplemented with horse sera, thallium acetate, and penicillin) and incubated at 37°C for two weeks under microaerophilic conditions according to Carter (1990) and Quinn et al. (1994).

Antibiotic susceptibility test

For the antibiotic susceptibility test, colonies from the Columbia blood agar medium were suspended in 2 ml of sterile saline and density of these suspensions was adjusted to McFarland Opacity Standard No; 0.5. The bacterial suspensions were inoculated onto Mueller-Hinton agar with the dry cotton swabs. The antibiotic disks, containing the antibiotics were dispensed on the surface of the medium and incubated aerobically at 37ºC for 18 h. Antimicrobial sensitivity was tested by the disk diffusion method of Bauer et al. (1966) on Mueller Hinton Agar (Oxoid) and performed according to Clinical and Laboratory Standards Institute (CLSI, 2013). The following antibacterial disks (Oxoid) were used: Penicillin G (10 µg, P), Amoxicillin (10 µg, AML), Amoxicillin + Clavulanic acid (30µg, AMC), Enrofloxacin (5µg, ENR), Gentamicin (10µg, CN), Oxytetracycline (30 µg, T) and Erythromycin (15µg, E). The results were recorded as resistant, intermediate or susceptible by the measurement of the inhibition zone diameter according to the interpretive standards of CLSI (2013). *S. aureus* ATCC 25923 and *S. epidermidis* (ATCC 12228) were used as control strain for the antibiotic susceptibility tests.

Molecular Diagnosis

PCR (Polymerase Chain Reaction) analyses were used for confirmation of biochemical identification of microorganisms. For the PCR analyses, *S. aureus* (ATCC 25923) and *S. epidermidis* (ATCC 12228) from department collection, and *Mycoplasma bovis* ATCC 25025 DNA (Dr. Jessie Trujillo, IOWA State University of Science and Technology, College of Veterinary Medicine, Department of Veterinary Microbiology and Preventive Medicine) were used as positive control DNA.

A loopful of bacterial cells from pure subculture of isolates was suspended in 1 ml of sterile PBS (Phosphate-Buffered Saline, pH 7.4) and centrifuged at 5,000 x g for 5 min. The pellets were then resuspended in 300 µl of TE buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA) and a nucleic acid extraction was implemented according to the method of Sambrook and Russell (2001). The properties of the

primers are shown in Table 1.The simplex PCR protocols and procedures were carried out according to their references. After amplification, ten microliters of each amplification reaction mixture was analysed by electrophoresis performed with a 1.5% (wt/vol) agarose gel stained with ethidium bromide (0.7 µg/ml). After migration with 160 volts for 30 minute, amplification products were visualized under ultraviolet light.

Table1. Properties of primers used in the study

Agent (Target Gene)	Primer Name	Primer Sequences	Reference
Staphylococcus spp. (*16s rRNA*)	Staph294-318	5′-GCCGGTGGAGTAACCTTTTAGGAGC-3′	Cantekin et al. 2013
	Staph1522-1540	5′-AGGAGGTGATCCAACCGCA-3′	
S. aureus (*Coa* gene)	Coa 1	5'- GCTTCTCAATATGGTCCGAG-3'	Cantekin et al. 2013
	Coa 1	5'- CTTGTTGAATCTTGGTCTCGC-3'	
E. coli (23S r RNA)	Eco 2083	5'-GCTTGACACTGAACATTGAG-3'	Riffon et al. 2001
	Eco 2745	5'-GCACTTATCTCTTCCGCATT-3'	
S. agalactiae (16S rRNA gene)	Sag 40	5'-CGCTGAGGTTTGGTGTTTACA-3'	Riffon et al. 2001
	Sag 445	5'-CACTCCTACCAACGTTCTTC-3'	
S. dysgalactiae (16S rRNA gene)	Sdy 105	5'-AAAGGTGCAACTGCATCACTA-3'	Riffon et al. 2001
	Sdy 386	5'-GTCACATGGTGGATTTTCCA-3'	
S. parauberis (23S rRNA gene)	Spa 301	5'-GCGACGTGGGATCAAATACT-3'	Riffon et al. 2001
	Spa 1219	5'-TACCATTACCTCTAAAGGTA-3'	
S. uberis (23S rRNA gene)	Sub 302	5'-CGAAGTGGGACATAAAGTTA-3'	Riffon et al. 2001
	Sub 396	5'-CTGCTAGGGCTAAAGTCAAT-3'	
M. bovis (membrane lipoprotein P81 gene)	Mb 1113–1133	5′-TATTGGATCAACTGCTGGAT-3′	Foddai et al. 2005
	Mb 1542–1560	5′-AGATGCTCCACTTATCTTAG-3′	
T. pyogenes (Plo gene)	Plo1	5'-GGCCCGAATGTCACCGC-3′	Billington et al. 2002
	Plo2	5'-AACTCCGCCTCTAGCGC-3′	
Candida spp. (rRNA gene)	Cab1	5'-TATTAAAGTTGTTGCAG-3'	Niesters et al. 1993
	Cab2	5'-CCTGCTTTGAACACTCTAATTT-3'	

RESULTS

Bacterial isolates were obtained from 28 (12.73%) of the 220 milk samples. In two samples (Sample No. 23 and 25) obtained from goats in the Yayladagi region, mixed culture were isolated. Thirty strains were isolated from these samples. Among these 30 isolates, 15 (50%) coagulase-negative *Staphylococci* (CNS), 8 (26.67%) *S. aureus*, 5 (16.67%) *S. uberis* and 2 (6.67%) *Escherichia coli* were identified. No yeast, fungi or *Mycoplasma* spp. was isolated from the samples. The identifications of isolates were confirmed by PCR from direct culture of these microorganisms. The highest resistance was found against penicillin and amoxicillin, with 21 (70%) and 19 (63.33%) isolates resistant, respectively. The isolates were also highly sensitive to gentamicin (*n*=30, 100%), enrofloxacin (*n*=28, 93.3%), oxytetracycline (*n*=28, 93.3%) and amoxicillin + clavulanic acid (*n*=27, 90%). The results of identification and antibiotic susceptibility of each strain are shown in Table 2.

DISCUSSION

In this Study, bacterial isolates were obtained from 28 (12.73%) of the 220 milk samples. Among these 30 isolates, 15 (50%) were CNS, 8 (26.67%) were *S. aureus*, 5 (16.67%) were *S. uberis* and 2 (6.67%) were *E. coli*. Among the isolated organisms in this study, *CNS* were the most prevalent microorganism. Similarly, White and Hinckley (1999) reported that the most prevalent agents in goat mastitis were non-haemolytic *Staphylococcus* spp. (*n*=406, 38.2%), *S. aureus* (*n*=117, 11.0%), *Streptococcus* spp. other than *S. agalactiae* (*n*=43, 4.1%), *E. coli* (*n*=17, 1.6%) and *Pseudomonas* spp. (*n*=13, 1.2%). Aydin et al. (2009) detected pathogens in 60 samples and a prevalence of sub-clinical mastitis of 8.6% in 700 milk samples obtained from clinically healthy udder halves. They reported that CNS were the predominant organisms isolated (50%), followed by *Streptococcus* sp. (15%), *S. aureus* (11.7%) and other pathogens (23.3%). Ilhan et al. (2011) used classical bacteriological culture methods in a study of milk samples obtained from 148 goats. The bacteriological cultures were positive in 69 (46.6%) of the 148 samples. The bacterial strains isolated from the milk samples were 42 (60.8%) CNS, 11 (15.9%) *Staphylococcus aureus*, 11 (15.9%) *E. coli*, 2 (2.9%) *Corynebacterium* spp., 1 (1.4%) *Streptococcus* spp., 1 (1.4%) *C. pseudotuberculosis* and 1 (1.4%) *Aeromonas* sp. They concluded that CNS was the most frequently isolated bacterium in goat milk from animals with sub-clinical mastitis. Islam et al. (2011) also found that CNS were the most prevalent microorganisms in goat mastitis (*n*=52, 57.78%), followed by *S. aureus* (*n*=4, 4.44%), *Streptococcus* sp. (*n*=4, 4.44%), *Bacillus* sp., (*n*=3, 3.33%), *E. coli* (*n*=5, 5.55%) and unidentified Gram-negative bacteria (*n*=14, 15.55%). On the other hand, some studies (Ciftci et al. 1996; Isnel and Kirkan 2012, Najeeb et al. 2013) found coagulase-positive *Staphylococci* were the most prevalent microorganisms. Ciftci et al. (1996) reported a sub-clinical mastitis prevalence of 9% (45 milk samples) in 500 goats. In their study, coagulase-positive *Staphylococci* (38.2%) were the most prevalent

microorganisms, followed by CNS (11.8%), *Corynebacterıum* spp. (23.5%), *E. coli* (14.7%), yeast (5.9%) and *Flavobacterium* spp. (5.9%). Isnel and Kirkan (2012) isolated 102 (67.1%) microorganisms from 152 milk samples from hair goats. In their study, *S. aureus* (*n*=71, 69.6%) was the most common isolate, followed by *S. epidermidis* (*n*=8, 7.8%), *S. intermedius* (*n*=5, 4.9%), *S. hyicus* (*n*=6, 5.9%), *Corynebacterium* sp. (*n*=3, 2.9%), *Klebsiella pneumoniae* (*n*=4, 3.9%), *Pseudomonas* sp. (*n*=2, 2.0%), *E. coli* (*n*=2, 2.0%) and *Mannheimia haemolytica* (*n*=1, 1.0%). Ali et al. (2010) found that prevalence of subclinical mastitis was 71 (13%) in the 543 goats. And, they reported that among the isolated microorganism, *S. aureus* was the most common microorganism (45.34%), followed by *Streptococcus* spp. (22.74%), *E. coli* (11.55%) and *Klebsiella* spp. (3.65%). Najeeb et al. (2013) reported bacterial growth in 90 of 200 milk samples (45%) and identified 146 strains in positive milk samples. Among these strains, the prevalence of *S. aureus* (61.64%) was the highest, followed by *E. coli* (10.96%), *Streptococcus* spp. (9.59%), *Pseudomonas* spp., *Bacillus* spp. (6.85%) and *Corynebacterium* spp. (4.11%). In another study conducted in Hatay, Dogruer et al. (2013) reported bacterial growth in 41.4% of 200 CMT-positive milk samples from 505 goats. Among these strains, the most common microorganisms were CNS (51.1%), followed by coagulase-positive *Staphylococci* (20.4%), *Streptococcus* spp. (8%), *Bacillus* spp. (5.7%), *E. coli* (4.5%), *Corynebacterium* spp. (3.4%), *Pseudomonas* spp. (2.3%) and *Acinetobacter* spp. (2.3%). These differences might be because of milking hygiene (hygiene of parlour and the milker's hand), bedding material, climatic differences and the usage or not of post-milking teat dipping.

Table 2. Identification and antibiotic susceptibility of each isolated strain.

Samples Origin		**Identifications**	**Results of Antibiogram**						
YAYLA DAĞI			***ENR***	***AMC***	***P***	***AML***	***E***	***CN***	***OT***
3	G1	*S. uberis*	S	S	**R**	**R**	S	S	S
18	G2	*CNS*	S	S	S	S	S	S	S
22	G3	*S. aureus*	S	S	S	S	S	S	S
23	G4	*S. aureus*	S	S	**R**	**R**	**R**	S	S
23	G5	*S. uberis*	S	S	**R**	**R**	S	S	S
25	G6	*CNS*	S	S	**R**	**R**	**R**	S	S
25	G7	*S. uberis*	S	S	**R**	**R**	**R**	S	S
26	G8	*CNS*	S	S	**R**	**R**	S	S	S
27	G9	*S. aureus*	S	S	S	S	S	S	S
28	G10	*CNS*	S	S	S	**R**	S	S	S
31	G11	*CNS*	**R**	S	**R**	**R**	**R**	S	S
64	G12	*CNS*	S	S	S	S	S	S	S
70	G13	*S. aureus*	S	**R**	**R**	**R**	S	S	S
HASSA									
2	G14	*CNS*	S	S	S	S	S	S	S
10	G15	*CNS*	**R**	S	S	S	S	S	S
37	G16	*CNS*	S	S	**R**	S	S	S	S
38	G17	*S. aureus*	S	S	**R**	**R**	S	S	S
39	G18	*S. aureus*	S	S	S	S	S	S	S
REYHANLI									
14	G19	*CNS*	S	S	**R**	**R**	**R**	S	S
15	G20	*S. uberis*	S	S	**R**	**R**	S	S	S
20	G21	*S. aureus*	S	S	**R**	**R**	**R**	S	S
ALTINÖZÜ									
2	G22	*S. uberis*	S	S	**R**	**R**	S	S	S
11	G23	*S. aureus*	S	S	**R**	S	S	S	S
35	G24	*CNS*	S	S	**R**	**R**	S	S	S
37	G25	*CNS*	S	S	**R**	**R**	S	S	S
40	G26	*CNS*	S	S	**R**	S	S	S	S
50	G27	*CNS*	S	S	**R**	**R**	S	S	S
54	G28	*E. coli*	S	**R**	**R**	**R**	**R**	S	**I**
57	G29	*E. coli*	S	**R**	**R**	**R**	**R**	S	**I**
80	G30	*CNS*	S	S	S	S	S	S	S
		Resistant (%)	2 (6.67)	3 (10)	21 (70)	19 (63.33)	8 (26.67)	-	-
		Intermediate (%)	-	-	-	-	-	-	2 (6.67)
		Sensitive (%)	28 (93.3)	27 (90)	9 (30)	11 (36.67)	22 (73.33)	30 (100)	28 (93.3)

In this study, the highest antibiotic resistance was found against penicillin (*n*=21, 70%) and amoxicillin (*n*=19, 63.33%). The isolates were also highly sensitive to gentamicin (*n*=30, 100%), enrofloxacin (*n*=28, 93.3%), oxytetracycline (*n*=28, 93.3%) and amoxicillin plus clavulanic acid (*n*=27, 90%). Ali et al. (2010) reported similar results. In their study, the isolated strains were sensitive to gentamicin (96.15%), with the greatest resistance found against penicillin G (42.13%). Aydin et al. (2009) reported that most strains that were resistant to penicillin were also sensitive to gentamicin and enrofloxacin. Dogruer et al. (2013) reported that application of intramammarian ampicillin dicloxacillin with intramuscular amoxycillin clavulanic acid in goats with subclinical mastitis was very effective for therapy. Isnel and Kirkan (2012) found that isolates were susceptible to amoxicillin plus clavulanic acid (100%) and resistant to penicillin (100%). Najeeb et al. (2013) recorded the highest resistance against penicillin (58.69%). In their study, most strains were also sensitive (83.33%) to amoxicillin plus clavulanic acid. Dogruer et al. (2013) reported that isolates were resistant to penicillin (77.7%), oxytetracycline (53.3%), gentamicin (53.3%), amoxicillin (51.1%), trimethoprim-sulfamethoxazole (33.3%), enrofloxacin (17.7%), amoxicillin plus clavulanic acid (11.1%), kanamycin-cefuroxime (0.06%) and cephalothin (0%).

CONCLUSION

In conclusion, this study identified goat mastitis agents in Hatay and investigated their antibacterial resistance. *Staphylococci*, especially CNS, were the most prevalent microorganisms in goat mastitis. These can be harmful to goat milk production by causing sub-clinical mastitis. The high prevalence of penicillin resistance in mastitis isolates is well known. The acquisition and spread of beta-lactamase activity among microorganisms is very easy and rapid. The use of penicillin containing the beta-lactamase inhibitor, such as clavulanic acid, can help to combat beta-lactamase activity. The use of beta-lactamase-inhibitor with penicillin can also be a useful strategy. High sensitivity to tetracycline in this study may be due to more less using this antibiotic in goat mastitis treatment. Periodical studies and constant monitoring of antibiotic resistance might be beneficial for treatment and prevention strategies and used to manage the acquisition of antibiotic resistance.

REFERENCES

Ali Z, Muhammad G, Ahmad T, Khan R, Nazet S (2010). Prevalence of Caprine Sub-Clinical Mastitis, its Etiological Agents and their Sensitivity to Antibiotics in Indigenous Breeds of Kohat, Pakistan. *Pak J Life Soc Sci*, 8(1), 63–67.

Anonym (2016). Hatay ili Tarımsal Yatırım Rehberi, http://www.tarim.gov.tr/SGB/TARYAT/Belgeler/il_yatirim_rehberleri/hatay.pdf Erişim Tarihi: 06 Mart 2016.

Aydin İ, Kav K, Çelik HA (2009). Identification and antimicrobial susceptibility of subclinical mastitis pathogens isolated from hair goats' milk. *J Anim Vet Adv*, 8, 1086-1090.

Bauer AU, Kirby WM, Sherris JC, Tack M (1966). Antibiotic susceptibility testing by a standardized single disc method. *J Clin Pathol*, 45, 493-494.

Bergonier DB, De Crémoux R, Rupp R, Lagriffoul G, Berthelot X (2003). Mastitis of dairy small ruminants. *Vet Res*, 34, 689–716.

Billington SJ, Post KW, Jost BH (2002). Isolation of Arcanobacterium (Actinomyces) pyogenes from cases of feline otitis externa and canine cystitis. *J Vet Diagn Invest*, 14(2),159-162.

Boyazoglu J, Hatziminaoglou I, Morand-Fehr P (2005). The Role of the Goat in Society: Past, Present and Perspectives for the Future. *Small Rum Res,* 60, 13-23.

Cantekin Z, Saidi R, Solmaz H, Ergun Y (2014). A Duplex PCR for Detection of *S. aureus* and *Staphylococcus* spp. from Culture and Bovine Milk Samples. *YYU Vet Fak Derg*, 2014, 25 (1), 11 – 13.

Carter GR (1990). Isolation and identification of bacteria from clinical specimens. In: Carter GR, Cole JR. (Eds.) Diagnostic Procedures in Veterinary Bacteriology and Mycology. 5th edition, Academic Press Inc. San Diego, California.

Çiftci MK, Berkin Ş, Erer H, Erganiş O, Kıran MM, Hatipoglu F, Saglam YS (1996). Keçi mastitisleri üzerinde patolojik ve bakteriyolojik çalişmalar. *Vet Bil Derg*, 12(2),105–114.

CLSI (2013). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement. CLSI document M100-S23. Wayne, PA: Clinical and Laboratory Standards Institute.

Dellal I, Dellal G (2005). Türkiye Keçi Yetiştiriciliğinin Ekonomisi. Süt Keçiciliği Ulusal Kongresi, 26-27 Mayıs, İzmir.

Dogruer G, Saribay MK, Aslantas O, Ergun Y, Aslantas O, Demir C, Ates CT (2013). Treatment of subclinical mastitis in Damascus goats during lactation. *Small Rum Res* 90,153-155.

Dogruer G, Saribay MK, Aslantaş Ö, Kireççi E, Ergün Y, Ülkü A, Demir,C (2013). Keçilerde subklinik mastitislerde prevalans, etiyoloji ve mikroorganizmaların antibiyotik duyarlılıkları V.Veteriner Doğum ve Jinekoloji Kongresi, 31 Ekim – 3 Kasım, Antalya, 2013.

Foddai A, Idini G, Fusco M, Rosa N, de la Fe C, Zinellu S, Corona L, Tola S (2005). Rapid differential diagnosis of Mycoplasma agalactiae and Mycoplasma bovis based on a multiplex-PCR and a PCR-RFLP. *Mol Cell Probes*, 19(3), 207-212.

Günlü A, Alaşahan S (2010). Türkiye 'de Keçi Yetiştiriciliği ve Geleceği Üzerine Bazı Değerlendirmeler (Evaluations on the Future of Goat Breeding in Turkey). *Vet Hekim Der Derg,* 81(2), 15–20.

IDF (1985). International Dairy Federation, Milk and Milk Products: Guidance on Sampling. International Dairy Federation, Standard 50B, Brussels, Belgium.

Ilhan Z, Taşal I, Sagcan S, Solmaz H (2011). Subklinik Mastitisli Keçi Sütlerinden Aerobik Bakterilerin İzolasyonu Isolation of Aerobic Bacteria from Goat Milk with Subclinical Mastitis. *YYU Vet Fak Derg*, 22(2), 89–91.

Islam MA, Samad MA, Anisur Rahman AKM (2011). Bacterial Pathogens and Risk Factors Associated With Mastitis in Black Bengal Goats in Bangladesh. B*angl J Vet Med*, 9(2), 155–159.

Isnel NB, Kırkan S (2012). Isolation of Microorganisms from Goats with Subclinical Mastitis and Detection of Antibiotics Susceptibility. Subklinik Mastitisli Keçilerden Mikroorganizmaların İzolasyonu ve Antibiyotiklere Duyarlılıklarının Araştırılması. *Animal Health Prod and Hyg*, 1(2), 106 – 112.

Najeeb MF, Anjum AA, Ahmad MUD, Khan HM, Ali MA, Sattar MMK (2013). Bacterial etiology of subclinical mastitis in dairy goats and multiple drug resistance of the isolates. *J Anim Plant Sci*, 23(6),1541–1544.

Niesters HGM, Goessens WHF, Meis JFMG, Quint WGV (1993). Rapid, polymerase chain reaction-based identification assays for Candida species. J Clin Microbiol, 31,904–910.

Poutrel B, De Crémoux R, Ducelliez M, Verneau D (1997). Control of intramammary infections in goats: impact on somatic cell counts. J Anim Sci, 75,566-570.

Quinn PJ, Carter ME, Markey BK, Carter GR (1994). Clinical Veterinary Microbiology. Mosby-Year Book Europe Limited, Lynton House, London WC1H9LB, England.p. 209-236.

Riffon R, Sayasith K, Khalil H, Dubreuil P, Drolet M, Lagace A (2001). Development of a Rapid and Sensitive Test for Identification of Major Pathogens in Bovine Mastitis by PCR. J Clin Microbiol, 39(7), 2584–2589.

Sambrook J and Russell W (2001). Molecular cloning: a laboratory manual (3rd ed.), Cold Spring Harbor Press, New York.A8.9-A8.10. 2049-2050.

White EC, Hinckley LS (1999). Prevalence of mastitis pathogens in goat milk. *Small Rum Res*, 33,117-121.